JOURNAL OF DAIRY SCIENCE

JANUARY, 1957

In this Issue:

A.D.S.A. Annual Meeting Announcement

New Journal Procedures

News Items

Twelve Articles on Original Research

Officers and Committees of the Association

Abstracts of Literature

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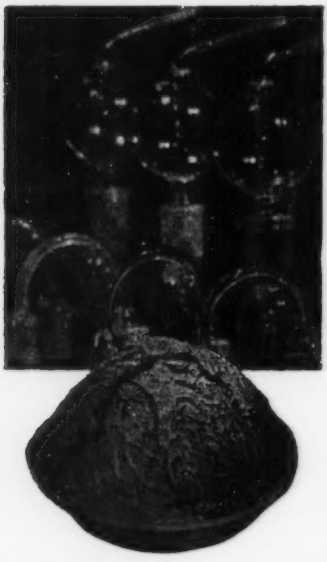

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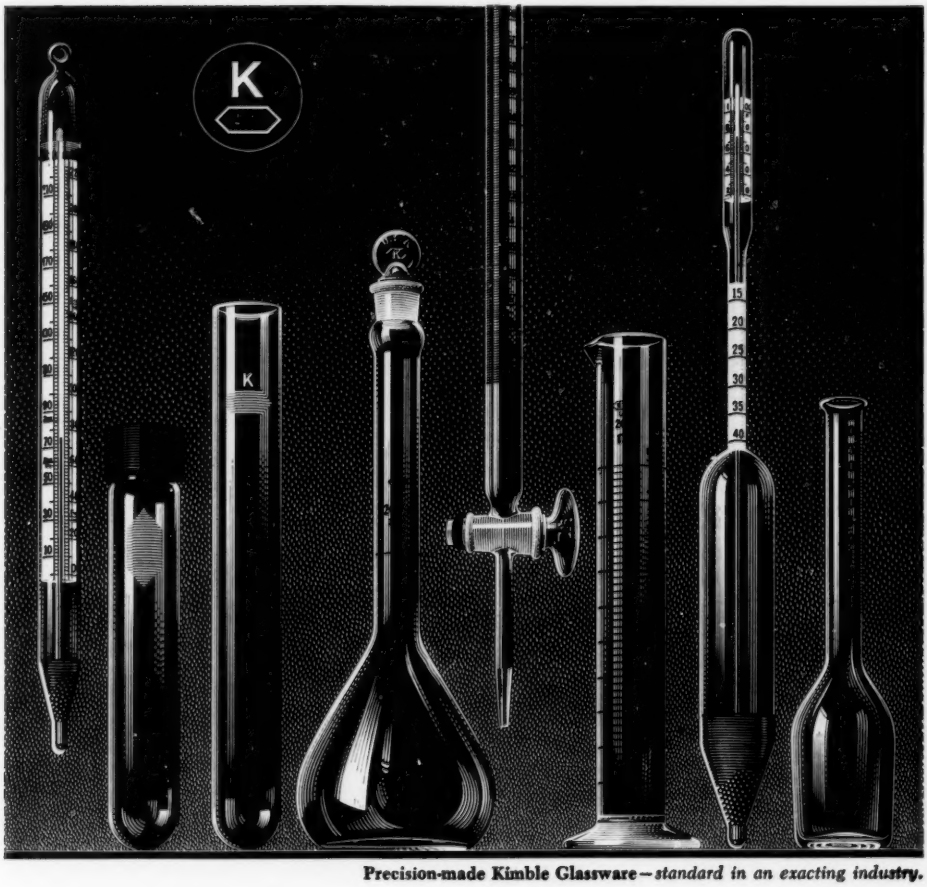

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Manuscripts for publication in the Journal of Dairy Science should be submitted to the editor, E. O. Herreid, at the address above. See January, 1954, issue, page 103, for information concerning the preparation of manuscripts, or send for reprint, "Information for Authors."

Advertising

Advertising plates, copy, insertion orders, contracts, and requests for information relating to advertising in the Journal should be addressed to The Garrard Press, 119-123 West Park Ave., Champaign, Ill.

Nonmember Subscriptions

Nonmember subscriptions are $15.00 per volume in the United States and Canada; $15.50 in all other countries, net and postpaid. New subscriptions and renewals begin with the first issue of the current volume. Renewals should be sent in by December 1 of the current year for the following calendar year to avoid a break in the series. Subscriptions should be sent to the Journal of Dairy Science, in care of The Garrard Press, 119-123 West Park Avenue, Champaign, Illinois.

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Memberships

Membership dues in the Association are $10.00 a year and should be sent to H. F. Judkins, Secretary-Treasurer, 32 Ridgeway Circle, White Plains, New York. Renewals should be made not later than December 1 each year.

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PEOPLE and EVENTS
in the Dairy Science World

Special A.D.S.A. Committee Announces New Journal Procedures

A special committee was appointed in June, 1956, by the Executive Board of the American Dairy Science Association to study the handling of our editorial services. Members of this committee — President C. F. HUFFMAN, W. M. ROBERTS, and W. V. PRICE — completed their assignment in September, 1956, when the Executive Board accepted their recommendations.

As a result of this action a new system of handling editorial work comes into operation with this issue of the Journal, when E. O. HERREID succeeds PAUL H. TRACY as Editor-in-Chief of the *Journal of Dairy Science*.

In recent years the editorial work of preparing the Journal for printing has grown steadily. The popular new departments recently introduced by Paul H. Tracy require additional planning, correspondence, and editorial supervision. It has become apparent to members of the Journal Management Committee and the Executive Board that these editorial responsibilities have outgrown the services of only a part-time editor and his assistants. Reorganization and reassignment of editorial responsibilities have been made under the new system.

The new changes assign all routine steps of preparing the Journal for the printer to a professional staff of The Garrard Press. The screening of all papers, articles, and abstracts remains in the hands of our Editor, his assistant, W. O. NELSON (in charge of Abstracts), and the 12 members of the Editorial Board.

The routine editorial services will be the direct responsibility of RICHARD J. DANDENEAU of The Garrard Press, Champaign, Illinois. Mr. Dandeneau obtained his B.A. degree at Ohio Wesleyan Univ., Delaware, and his M.S. in Journalism at the Univ. of Wisconsin, Madison. He also has taken post-Master's work in English and speech at Michigan State Univ., East Lansing, and at the Univ. of Illinois, Urbana.

Before joining the staff of The Garrard Press, Mr. Dandeneau did editorial work with several newspapers, was a member of the public relations staff at Michigan State Univ., and served as editor for the Bureau of Business Management and taught Business English at the Univ. of Illinois.

As each paper is approved for publication by our Editor-in-Chief, it will be given to Mr. Dandeneau and his staff to edit for style, punctuation, and basic grammatical construction before it goes to the printer. All proofs, as usual, will be sent to the author and our Editor-in-Chief for approval before printing.

Contributors of original papers, articles, and abstracts to the Journal will see no change in the routine of publication. News correspondents should send their material directly to Mr. Dandeneau at The Garrard Press, but even news items will be checked by Editor Herreid before publication.

In addition to these editorial services, The Garrard Press will handle nonmember subscriptions and work connected with advertising other than sales.

Details of all of these services were developed during many weeks of conferences and correspondence with The Garrard Press, other agencies, and Secretary H. F. JUDKINS before the program was finally recommended to the Executive Board for action. Other organizations involved in these discussions were: The American Society of Agronomy, The American Association of Cereal Chemists, The Olsen Publishing Company, and The Waverly Press. All were most helpful in aiding the committee in its study of the problem. Special thanks go to PAUL H. MANDT of The Olsen Publishing Company for conferring directly with the Executive Board and extending every possible aid to the committee.

The committee is especially grateful for the cheerful assistance of L. G. MONTHEY, executive secretary of The American Society of Agronomy, and R. J. TARLETON, business manager of The American Association of Cereal Chemists. Explorations of common interests with these men have been most stimulating. They and the organizations they represent have indicated to the officers of the A.D.S.A. more than a casual interest in closer cooperation. Eventually intersociety cooperation may make possible greater efficiency in serving members, and attractive economies in costs of business management for all groups concerned.

A.D.S.A. Annual Meeting Dates Set for June 26–29, 1957

The dates for the 1957 annual meeting of the A.D.S.A. have been set for June 26–29 by the host institution, Oklahoma A & M College, Stillwater. The meeting will coincide with Oklahoma's celebration of its 50th year of statehood.

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Plans for the meeting are being made by committee members, some of whom are shown above. Left to right are: MRS. C. L. NORTON, chairman of the women's committee; C. L. NORTON, head, Department of Dairying, Oklahoma A & M; OLIVER S. WILLHAM, president of Oklahoma A & M; S. D. MUSGRAVE, co-chairman of evening, women's, and children's committees; H. C. OLSON, chairman of the steering committee; and A. E. DARLOW, vice-president and dean of agriculture, Oklahoma A & M.

Balch Studying at V.P.I.

DONALD BALCH, instructor in the Animal and Dairy Husbandry Department, Univ. of Vermont, Burlington, is on leave of absence studying for his Ph.D. degree at Virginia Polytechnic Institute, Blacksburg. KENNETH CHAPMAN (B.S., Univ. of Maine, 1954; M.S., Univ. of Vermont, 1956) is taking his place.

News from Montana State

J. C. BOYD, associate professor, Univ. of Idaho, Moscow, has become head of the Dairy Industry Department at Montana State College, Bozeman. Dr. Boyd earned the B.S. degree in dairy manufacturing at Washington State College, Pullman, in 1939; the M.S. degree at Montana State in 1942; and the Ph.D. degree at Michigan State Univ., East Lansing, in 1955. He succeeds J. A. NELSON, who has served in the department for over 34 years, 29 as head of the department. Dr. Nelson will remain in the department, devoting full time to teaching and research.

J. O. TRETSVEN, extension dairyman, has retired from active duty. He served for 47 years, first as herdsman, later in teaching and research, and then as extension dairy specialist. He was the winner of the DeLaval Extension Dairyman Award for distinguished work at the 50th annual meeting of A.D.S.A. held at the Univ. of Connecticut, Storrs, in June, 1956.

The 21st Annual Dairy Industry Short Course was held at Montana State College, Nov. 12–14. Appearing on the program as guest instructors were: R. D. BOVEY, Klenzade Products, Inc.; C. D. DAHLE, professor emeritus, The Pennsylvania State Univ., University Park; C. C. GORHAM, Gold Medal Dairies; JAY C. HARRIS, Evergreen Northwest Breeders, Inc.; LLOYD R. HUNZAKER, Utah State Agricultural College, Logan; and S. A. ROBERT, JR., American Dairy Association. The 22nd Annual Short Course will be held Nov. 18–20, 1957.

Ohio State News Items

HOWARD A. HARTLEY has been appointed instructor in the Department of Dairy Technology, effective Jan. 1. His duties will be to manage the dairy plant.

Mr. Hartley received a B.S. degree from Purdue University, West Lafayette, Ind., in 1953, and an M.S. degree from the University of Idaho, Moscow, in 1956. For the past three years, he has been an instructor at Idaho.

DALE A. SEIBERLING has been granted a leave of absence to serve as a dairy engineering consultant to the International Cooperative Administration and the Technical Cooperative Mission to India.

Mr. Seiberling left the first of the year for a three-day stay in London en route to New Delhi, India. While in India, where he will spend about 10 weeks, he will assist with the development of equipment layouts and specifications for a pasteurizing and bottling plant for the Calcutta Milk Scheme, a spray-drying plant, several small dairies, and two agricultural college dairy plants.

Over 200 dairy plant fieldmen, sanitarians, and others interested in field problems attended five district field meetings in Ohio. Herd health, local dairy economic outlook, milk production efficiencies, quality milk, and producer-fieldman relationships were discussed.

The meetings were sponsored by the O.S.U. Department of Dairy Technology and the Ohio Dairy Products Association.

The high-school student recruitment program got under way at O.S.U. January 22 with a dinner meeting for high-school principals and counselors. Preceding the meeting, a tour of the facilities of the Department of Dairy Technology was conducted.

The program resulted from a meeting held in December by the Education Committee of the Columbus Milk Distributors Association on the O.S.U. campus.

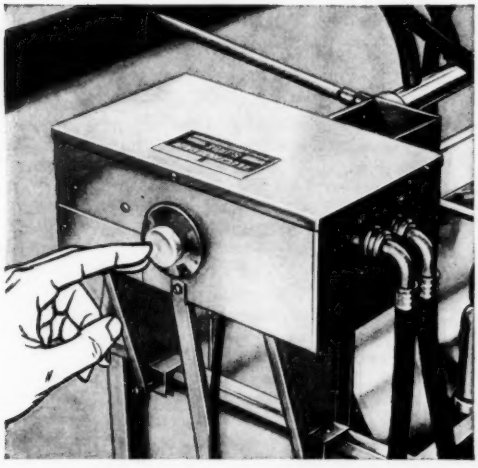
Prominent visiting speakers will participate in the 24th Annual Dairy Technology Conference at O.S.U., Feb. 5–8, according to preliminary program plans.

Speakers included are R. C. THOMSEN, University of Wisconsin, Madison; E. O. HERREID, University of Illinois, Urbana; R. DIETERICH, National Dairy Products Co.; J. CULLEN and C. HARPER, Beatrice Foods Co.; J. E. CRAFTS, Batavia Transportation Co.; K. KICKINSON,

C.I.P.

the ELECTROBRAIN push-button way

A while back, we ran a series of meetings in which the proper washing of bucket and pipe line milking machines was discussed in some detail. The meetings were well attended... but... they called for more time and man power than we could produce.



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A number of other specialists will also appear to discuss developments in the fields of butter, field work, market milk, engineering, ice cream, and condensed products.

The Ohio Dairy Products Association will hold its annual convention Feb. 18–20 at the Hotel Cleveland, Cleveland, O.

The date of the Milk Sanitarians Short Course at O.S.U. has been changed from March 11–15 to April 1–5.

Penn State News Items

During the past year the Dairy Science Department at The Pennsylvania State Univ., University Park, was privileged to have as a research associate DAVID A. FORSS, a research officer in the Dairy Research Section, Commonwealth Scientific and Industrial Research Organization of Australia.

Dr. Forss's visit here was made possible through a year's leave of absence. He was accompanied by his wife and daughter.

His principal research efforts at Penn State concerned identification of flavor compounds produced in milk by ionizing radiations. The use of gas chromatography and mass spectrometry were emphasized in this work which was supported by the Radiation Preservation Branch, Quartermaster Food and Container Institute for the Armed Forces, Chicago, Ill.

The Forsses made several trips in this country — to such points of interest as Massachusetts Institute of Technology, Cambridge; University of Illinois, Urbana; Quartermaster Research and Development Command, Natick, Mass.; Philip Morris Research Laboratories, Richmond, Va.; the A.D.S.A. annual meeting, Storrs, Conn.; and the American Chemical Society meeting, Atlantic City, N. J.

Mr. Forss and his family left Nov. 1 to return to Australia via England and the Cape of Good Hope. En route he is visiting laboratories concerned with flavor research and gas chromatography in England and other parts of Europe.

Mr. Forss is widely known for his research on the identification of carbonyl compounds associated with oxidized flavor in milk. It was a most stimulating experience to have him and his family in the dairy group at University Park.

"Miracle of Milk" Reprints Still Available

A limited number of reprints of "The Miracle of Milk" (*Journal of Dairy Science*, Sept., 1955) by L. L. RUSOFF, Louisiana State Univ., Baton Rouge, are still available in the Editor-in-Chief's office. Orders may be directed to E. O. HERREID, Department of Food Technology, 101 Dairy Manufactures, Univ. of Illinois, Urbana. Cost of the reprint is 9 cents.

Dairy Meeting to Be Held in New Georgia Conference Center

The Ice Cream Short Course and Food Handlers Conference will be held Feb. 18–21, at the Univ. of Georgia, Athens.

The meeting will be held in the new Georgia Center for Continuing Education. This Center has 26 conference rooms that will seat 786 people. The auditorium will accommodate 444 people and it has a stage equipped for television performances. There are dining-room facilities for 950 people, bedrooms for 296, and parking for 833 automobiles. Beneath the auditorium is an exhibit hall.

This fine center for adult education was made possible by grants from the State of Georgia and from the W. K. Kellogg Foundation, Battle Creek, Mich.

Michigan State to Host Feb. Dairy Engineering Meet

More profit through better engineering will be the theme of the Annual National Dairy Engineering Conference to be held Feb. 26–27 in the Kellogg Center at Michigan State Univ., East Lansing.

Timely subjects to be discussed are new developments in pasteurization and equipment, the packaging and distribution of milk, plant expansion and handling materials, and plant design and maintenance.

In addition to prominent speakers from industry, appearing on the program will be C. W. HALL and J. J. PELUG, Department of Agricultural Engineering, Michigan State Univ. G. M. TROUT, Dairy Department, Michigan State Univ., will moderate a panel of experts on topics discussed during the conference.

Flavor, Sanitation, Automation Discussed at Kentucky Meeting

Milk product flavors, sanitation, and automation were the subjects discussed at the Univ. of Kentucky's Fourth Annual Dairy Manufacturing Short Course held recently at Lexington.

Some 90 industrial people heard banquet speaker, PROF. B. E. GOODALE of Clemson Col-

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Specifications	DynaFOS	Soft or Colloidal Phosphate	Curacao Island Phosphate	Bone Meal
1 Phosphorus Content	18.5%	9.0%	14.0%	13%
2 Dynamic (Biological) Value	100	Less than 25	70	70-100
3 Lbs. of Usable Phosphorus Per Ton	370 lbs.	Less than 45.0 lbs.	196 lbs.	182-260 lbs.
4 Maximum Fluorine Content	1,200 ppm	15,000 ppm	5,000 ppm	600-8,760 ppm

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lege, Clemson, S. C., emphasize the importance of flavor by stating, "Flavor influences the acceptability of nearly everything that passes the lips. . . ."

He added that in this great scientific age dairymen are not as progressive in controlling flavor as are their competitors in other food industries.

Clinics were conducted on ice cream by W. M. ROBERTS, North Carolina State College, Raleigh; on buttermilk and cottage cheese by M. F. HALES, Chr. Hansen's Laboratories; and on cheese by H. E. MEISTER, Acting Chief of Inspection and Grading Branch, USDA, Washington, D.C.

The Short Course was sponsored jointly by the Dairy Section of the University and the Kentucky Dairy Products Association.

Aschbacher Joins North Dakota Staff

A new addition to the staff of North Dakota Agricultural College, Fargo, is PETER W. ASCHBACHER, who comes to North Dakota as assistant professor of dairy husbandry and assistant dairy husbandman of the North Dakota Agricultural Experiment Station.

He replaces T. L. FORSTER, who has joined the staff of Washington State College, Pullman.

Mr. Aschbacher was born and reared on a dairy farm near Ashville, Wis., and received his B.S. and M.S. degrees from the Univ. of Wisconsin. He expects to complete his Ph.D. at Wisconsin in the physiology of reproduction.

Louisiana State News

W. J. A. PAYNE, Animal Husbandry Office, British Fiji Civil Service, who is in the U.S. on a Commonwealth Fund Fellowship, will make his headquarters at Louisiana State Univ., Baton Rouge.

Mr. Payne, who will be in the U.S. for a year, has chosen L.S.U. as headquarters because he is primarily interested in climatology, especially the heat tolerance phase. His appointment as a visiting lecturer will permit him to visit such classes and laboratories as he may choose.

N. J. MATHERNE, formerly county agent of Washington Parish, has become an associate extension dairyman at L.S.U. He replaces L. L. FULMER, who has returned to county agent work.

F. R. LANDAGORA has joined the staff of the Department of Animal Industry, University of Puerto Rico, where he will do research and teaching in animal nutrition.

Mr. Landagora, a native of the Philippine Islands, received his B.S. degree from Utah State Agricultural College, Logan, and his M.S. degree from Oklahoma A & M College, Stillwater. He was working toward his Ph.D. degree at L.S.U. when he accepted the position in Puerto Rico.

The Weigh-a-Day-a-Month Plan of milk and feed recording on dairy farms is getting a start in Louisiana. So far, 10 farmers are participating in this program; and because this service is very inexpensive and entails a minimum of record-keeping there is a growing interest among dairy farmers. The one-day records are sent to L.S.U. for computation on IBM machines.

It is not expected that the Weigh-a-Day-a-Month Plan will displace D.H.I.A. associations; but rather that it will supplement them where farmers have been unable to form organizations and carry on more complete record-keeping.

Consumer milk-buying habits are changing, according to reports from the New Orleans area. Homogenized milk, paper containers, larger-sized containers, and buying from retail stores are all gaining in popularity. Door-to-door delivery is losing out to the specialized dairy stores and groceries.

Judging Contest Held at Clemson

Judging teams from Mississippi, Texas Tech, and Maryland took top honors at the Southern Regional Dairy Products Judging Contest held recently at Clemson College, Clemson, S.C.

Banquet speaker was J. W. JONES, director of agricultural teaching at Clemson, who spoke on the topic, "The Dairy Industry in the Present Economy."

Superintendent of the contest was F. W. WARREN, North Carolina State College, Raleigh. A sidelight of the contest was a trip for the participants through Clemson's Agricultural Center, including the new dairy plant.

Reeves Joins Clemson Staff

CALVIN B. REEVES has been named extension dairy specialist for the Extension Service at Clemson College, Clemson, S.C.

Mr. Reeves received a B.S. degree in dairying from Oklahoma A & M College, Stillwater, in 1942, and an M.S. in dairy technology from The Ohio State Univ., Columbus, in 1947. He served for a time as assistant professor of dairy husbandry at New Mexico A & M College, Mesilla Park; and he has had practical experience in dairy plant operation in Philadelphia, Pa. While at Clemson, Mr. Reeves will devote his time to milk-processing plants including ice cream, butter, and evaporating plants.

News Items from Cornell

R. H. BARNES, former director of biochemistry at Merck, Sharp, and Dohme Research Laboratories, has been named dean of the School of Nutrition at Cornell Univ., Ithaca, N. Y.

He succeeds L. A. MAYNARD, who directed the school from its founding and who had taught at Cornell since 1915. Mr. Maynard retired in 1955.

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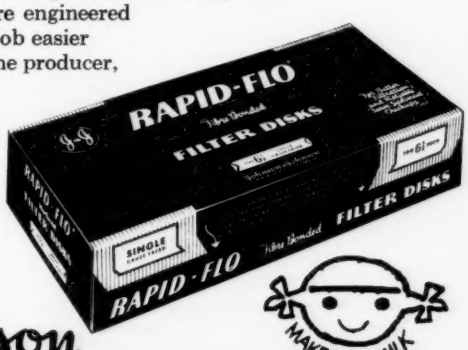

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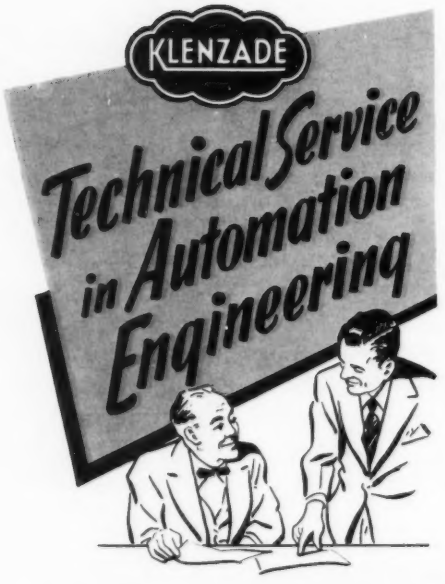

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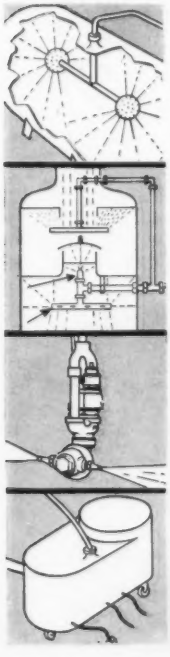

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Mr. Barnes received his Ph.D. in physiological chemistry at the Univ. of Minnesota, St. Paul, in 1940, and did his undergraduate work at San Diego Teachers College, San Diego, Calif. He has also been a member of the faculty of Rutgers Univ., New Brunswick, N. J.

WILLIAM K. JORDAN, Department of Dairy Industry, will be on sabbatical leave in Sweden during the spring term and summer of 1957. He will serve with the Research and Development Department of Wedholms A/B in Nyköping, Sweden, a company which manufactures dairy equipment. While abroad, Mr. Jordan plans to visit dairy schools in Scandanavia, Scotland, and England.

B. L. HERRINGTON, Department of Dairy Industry, will spend his sabbatical leave during the spring and summer, 1957, at the Royal College of Veterinary Medicine, Copenhagen, Denmark, where he will serve as a lecturer. While in Scandanavia, he will also visit research centers throughout Europe.

Some recent developments in the manufacture and quality control of cottage cheese were discussed during a recent cheese clinic at Cornell. ERIK LUNDSTEDT of H. P. HOOD and Sons, Boston, Mass. proposed the formation of a national organization of soft cheese manufacturers. The group unanimously approved the idea and elected Mr. Lundstedt chairman of the organizing committee.

F.D.A. Promotes J. K. Kirk

J.KENNETH KIRK, a technical administrative officer in the Food and Drug Administration's Bureau of Enforcement, has been named chief of the F.D.A. Boston District. He succeeds F. LESLIE HART, who has retired from government work after 38 years of service.

Mr. Kirk began his career with the F.D.A. as a junior inspector in 1930 and did inspection work in New York, Buffalo, Pittsburgh, and Philadelphia. Since 1940 he has specialized in regulatory work on food in the Washington administrative offices. He is the holder of awards for service from the National Civil Service League and from the U.S. Department of Health, Education, and Welfare.

Retiring Mr. Hart will go to Rio de Janeiro, Brazil, S.A., as a consultant for the Pan American Sanitary Bureau, World Health Organization.

Grant Hartman Recovering from Train-Auto Accident

GRANT HARTMAN, chemist for the White House Milk Co., Inc., Manitowoc, Wis., is recovering from injuries suffered when his car was struck by a train Dec. 11, just outside of Port Washington, Wis.

Recent Deaths

MRS. I. W. RUPEL, wife of I. W. RUPEL, Texas A & M, College Station, a member of the Board of Directors of the American Dairy Science Association, passed away Dec. 9, 1956.

ARTHUR HARRIS, 75, president of Arthur J. Harris and Co., Chicago, died Dec. 6 in Presbyterian Hospital, Chicago. Funeral services were held Dec. 10.

FRANK B. ASTROTH, 63, one of the nation's leading dairymen, died of a heart attack at his Farmington, Minn., dairy farm Nov. 30, 1956.

During his career he was president of the American Jersey Cattle Club for three terms; a field representative, 1921-1931; and a director of the club from 1947-1950. He was active in the Dairy Shrine Club, Purebred Dairy Cattle Assoc., and the Minnesota Jersey Cattle Club.

Mr. Astroth was nationally known for the registered Jersey breeding stock sold from his Bryn Mawr farm.

Wisconsin Fieldmen Meet Feb. 7; Manufacturers to Convene April 2

The 1957 Annual Wisconsin Dairy Plant Fieldmen's Conference is scheduled for Feb. 7-8 at the Univ. of Wisconsin, Madison.

Some 15 sessions are scheduled for the two-day meeting on the following topics: How Our Changing Agriculture Affects the Fieldman; New Research for New Farm Problems; Making Fieldwork More Effective; What the Fieldman Should Know About the Mastitis Problem in 1957; The Urgency of Meeting Quality Requirements in 1957; Milk Flavors and Their Control; Practical Feeding Schedules to Control Feed Flavors; Intake Inspection; What Research Tells Us About Meters for Measuring Milk from Bulk Tanks; Practical Experiences in Milk Measurements; What Research Tells Us About the Performances of Bulk Tanks; What About 3A Standards for Farm Milk Handling Equipment; Making Reciprocal Inspection Work; and Getting People to Understand.

H. C. JACKSON, Univ. of Wisconsin Department of Dairy and Food Industries, is chairman of the Feb. 7 session; and BURDETTE FISHER, also of the Dairy and Food Industries Department, is chairman of the Feb. 8 session.

Also to be held on the Univ. of Wisconsin campus April 2-3 is the Annual Wisconsin Dairy Manufacturers' Conference. The program for this meeting is still in the planning stage.

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Milk Concentrates Is Topic of March USDA Conference

Milk concentrates will be the featured topic at a research conference to be held March 19-20, 1957, at the USDA Eastern Regional Research Laboratory, Philadelphia, Pa.

The two-day conference has been developed by the USDA in cooperation with directors of the Eastern Agricultural Experiment Stations. Papers will be given by representatives from governmental agencies, universities, and industry.

Topics to be discussed include "Factors Influencing the Stability of Milk Proteins," "Some Effects on the Process of Homogenization on Protein Stability," "Nonprotein Nitrogen Changes in Stored Evaporated Milk," "Studies in Atmospheric Oxidation of Fatty Acids," "Fatty Acids of Milk," "Dried Whole Milk: A New Physical Form," "Process Innovations Improve Fresh Milk Concentrate," and "Frozen Concentrated Milk."

Hazelton, North, Noznick Receive Beatrice Service Emblem Awards

Three A.D.S.A. members have received service emblem awards from the Beatrice Foods Co., Chicago.

They are JOHN F. HAZLETON, executive vice-president, who received a diamond pin for 35 years service with Beatrice; GERALD C. NORTH, director of the new products and materials department, who received a 20-year service award; and PETER P. NOZNICK, director of research, who received a 15-year service award.

Completed Theses

M.S. degree

M. C. BADAMI — The effect of supercentrifugation on the components of the milk lipase systems. Ohio State Univ., Columbus.

Ph.D. degree

J. R. NICHOLS — Superovulation and ova transplantation in the bovine. Univ. of Minnesota, St. Paul.

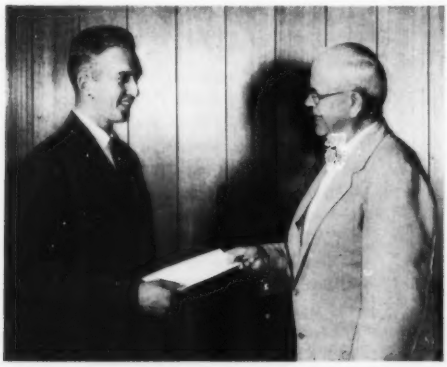
STUDENT CHAPTER NEWS

A section devoted to the activities of dairy students

E. L. THOMAS, *Editor*

Clemson College Student Honored

DIXON B. LEE, a dairy student of Clemson College, Clemson, S.C., was the recipient of the Milk Industry Foundation Agricultural Leadership Award from Zone II. The award is presented to a dairy senior for outstanding leadership abilities and achievements, and it consists of an all-expenses-paid trip to the Dairy Industries Exposition in Atlantic City, N.J., as a guest of the Milk Industry Foundation.



Professor B. E. Goodale of the Clemson College Dairy Department presents the Milk Industry Foundation Agricultural Leadership Award to Dixon Lee.

Twelve southern states comprise Zone II, and DIXON LEE is the second student from Clemson College to win this award.

Rutgers Dairy Science Club News

The Rutgers Dairy Science Club of Rutgers Univ., New Brunswick, N.J., held its eighth annual Fitting and Showmanship contest at the college farm on November 14 with four-year undergraduates and short course students competing. There were four classes in the competition — Brown Swiss cows, Jersey heifers, mature Jersey cows, and young Jersey bulls. JOHN SULLIVAN, a four-year student, won top individual honors and the four-year team topped the short course student team in competition for the Bartlett trophy. Prizes were donated by the Brown Swiss and Holstein Breed Associations.

A club exhibit, based on the theme "Typing and Herd Replacements," was set up at the annual Barn Hop. The main feature of this event is a square dance sponsored by the Agriculture School.

Texas Tech Club Holds Homecoming Breakfast

On November 3 at 7 A.M., the Dairy Industry Club at Texas Technological College held its 22nd Annual Homecoming Breakfast. The students served breakfast to seventy alumni. The judging team was presented, and their medals and trophies were displayed.

LETTER TO THE EDITOR

THANK YOU, EDITOR TRACY AND MRS. TRACY

The Journal Management Committee on behalf of the American Dairy Science Association wishes to acknowledge the great progress that the *Journal of Dairy Science* has made under the editorship of Dr. Tracy and his associate, Mrs. Tracy, and to express our sincere appreciation to them for their very excellent work and guidance during the past four years.

Editor Tracy obtained the services of a very competent editorial board from among the membership, and through their cooperative efforts our Journal ranks among the top scientific periodicals in the world in the editorial quality of its articles and in technical editing. Readability of the Journal has been increased by the addition of such sections as "People and Events in the Dairy Science World," "Our Industry Today," "Special Review Articles," "Student Chapter News," and brief abstracts at the beginning of each article. All of these improvements have made it possible to enlarge the membership in A.D.S.A. and to sell more advertising. Every member benefits from this improved condition of the Journal, because his publications get wider circulation; also, the resultant increased sale of advertising absorbs the rising cost of printing, thus making it unnecessary to raise membership dues.

Very few of us appreciate the tremendous amount of work involved in editing the Journal. Those who have had the responsibility of editing have had to sacrifice their week ends, holidays, and many evening hours. The financial reward has been so low that only those who are truly dedicated to the Journal and know its influence on the dairy industry have been willing to make this sacrifice. Oftentimes, we have been grossly negligent in expressing our appreciation for their generous contributions of time and talents. Editor and Mrs. Tracy have dedicated themselves to the Journal for the last four years and because of their foresight, energy, sound judgment, and vision, it has reached new heights of excellence and influence.

There is no way that we can adequately express our appreciation for the contributions that they have made to the Journal and thus to the Association, but we do want to take this opportunity to say "Thank you, Editor Tracy and Mrs. Tracy, for a job well done."

G. M. WERNER
D. H. JACOBSEN
W. M. ROBERTS, *Chairman*

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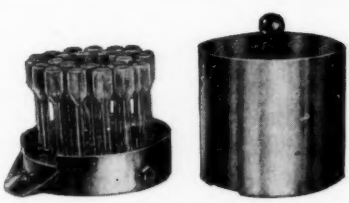

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FROM THE SECRETARY'S DESK

This is the time of year when your Membership Committee and your Secretary are doing their utmost to maintain and increase membership in our Association. A number of suggestions have been made to the Committee and, of course, reports are furnished them by the Secretary from time to time.

In addition to this, the Secretary does what he can by way of direct appeal. The names of representatives of companies visiting the Exposition at Atlantic City, N.J., last fall were published each day. These lists were studied and letters were mailed to some 400 companies. While we have received no avalanche of memberships or subscriptions, the response has been sufficient to justify the effort, and we have picked up a considerable number of two-year subscriptions by offering a copy of the Golden Jubilee issue as a bonus. We have suggested that the Membership Committee also make full use of this special offer.

As of this date, based on past experience, it would appear that we should reach the Membership Committee's goal of 2,200 for 1957. It appears that about 500 people who were members in 1956 will not have renewed by Jan. 1, 1957, but based on the 1955-56 experience this number will be eventually reduced to about 100. The membership should realize what this means in the way of clerical work for the Secretary as well as for The Garrard Press. To comply with our Constitution, the Secretary must send suspend cards to the printer for all who have not paid their dues by January 1. The printer must remove their Journal address plates from his file. As fast as their dues are paid after Jan. 1, reinstatement cards must be sent to the printer and he must put the address back in his active file. So, fellow members, don't be alarmed if your January issue is very late, if you are one who has not paid his 1957 dues by Jan. 1, 1957.

In September your Secretary secured the names of juniors, seniors, and graduate students majoring in dairying from most of the state universities, and by way of special appeal, sent a letter about mid-December to nearly 400 seniors and graduate students, pointing out the advantages of becoming an affiliate member.

The response has been very satisfactory and new affiliate member applications are being received daily. This would seem to indicate that some dairy department staff members must work a little harder in explaining the advantages of affiliate membership to their students. This is not said in the spirit of general criticism, but "if the shoe really fits, don't hesitate to put it on." — H. F. JUDKINS.

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INTERRELATIONSHIPS OF MICROORGANISMS IN CREAM. II. *LACTOBACILLUS CASEI, BACILLUS SUBTILIS,* AND *STREPTOCOCCUS LACTIS*[1]

L. G. HARMON[2] AND F. E. NELSON
Department of Dairy Industry, Iowa State College, Ames

Bacillus subtilis and Lactobacillus casei were inoculated individually and in combination with Streptococcus lactis into sterile cream which was incubated at different temperatures and analyzed for protein and fat decomposition, pH, growth and flavor changes. *Editor.*

Measurements of proteolysis by the tryptophan determination of Duggan *(8)* and water-insoluble acids (W.I.A.) by the Hillig *(11)* method have been used by regulatory agencies and the dairy industry in evaluating the quality of cream and butter. An investigation was performed to determine the amounts of tryptophan, W.I.A., and other deterioration values resulting from the growth of *Bacillus subtilis, Lactobacillus casei,* and combinations of these organisms with *Streptococcus lactis.*

The proteolytic activity of *S. lactis* in milk has been reported by numerous authors who were cited previously *(10)*. The proteolytic action of *L. casei* was investigated by Brandsaeter and Nelson *(5)*, who found maximum proteolysis of casein by cell-free extracts at pH levels of 6.0 to 7.0. Measurable proteolysis was produced at pH 4.5, but the rate was much below that at pH 6.5. Peterson *et al. (17)* encountered instances in which lactobacilli and streptococci in milk utilized more amino and ammonia nitrogen than they produced. Members of both genera continued to cause proteolysis after sugar destruction had ceased.

Several workers have reported on the proteolytic activity of bacilli isolated from milk. A number of spore-forming bacilli, including *B. subtilis,* studied by Lisk *(12)* produced progressive increases in ammonia nitrogen in sterile milk, with decomposition of milk protein proceeding beyond amino acids to form indol. Parfitt and Spitzer *(16)* found that a number of spore-forming bacilli produced the greatest protein hydrolysis in milk when the pH was near neutral. Berger *et al. (4)* studied the proteolytic activity of a number of organisms, including *B. subtilis* and reported that the optimum pH for the peptidase of *B. subtilis* was 8.0 to 9.0.

Baribo and Foster *(2, 3)* reported that each of nine strains of *S. lactis* studied produced a substance inhibitory to *L. casei.* At pH 5.4 to 4.8 the activity of the substance and its stability to heat increased. Most of the inhibitor was

Received for publication April 23, 1956.

[1] Journal Paper No. *J-2938* of the Iowa Agr. Expt. Station, Ames. Project No. 1050.

[2] Present address: Dairy Department, Michigan State University, East Lansing.

formed during the first 24 hours, but there was a continuous increase for 60 hours.

Marshall *(13)* and Cox and Whitehead *(7)* reported that when *B. subtilis* was added to a milk culture of *S. lactis* the lactic fermentation was stimulated. The stimulation was more evident at low incubation temperatures.

EXPERIMENTAL PROCEDURE

Proteolysis, lipolysis, organism populations, pH, and organoleptic grade resulting from the growth of *L. casei* and *B. subtilis*, individually and in combination with *S. lactis*, were determined in sterile cream incubated at various temperatures. Cream samples were prepared, inoculated, incubated, and analyzed according to procedures outlined previously *(10)*.

L. casei counts were made on the medium developed by Rogosa *et al. (18)*. Preliminary trials with three strains of *S. lactis* and three strains of *L. casei* demonstrated that this medium supported growth of *L. casei* and inhibited growth of *S. lactis*. Incubation was at 32° C. for 72 hours. When *S. lactis* and *L. casei* were grown as a mixed culture, *S. lactis* colonies were easily distinguished from *L. casei* after 48 hours at 32° C. on tryptone glucose beef extract milk (T.G.E.M.) agar (1).

B. subtilis populations were enumerated on a low-carbohydrate medium containing 0.3% beef extract, 0.5% peptone, and 1.5% agar. *S. lactis* colonies were inhibited on this medium. When *S. lactis* and *B. subtilis* were grown as mixed cultures on T.G.E.M. agar incubated at 32° C. for 48 hours, *S. lactis* colonies completely dominated the growth on the plates and were easily distinguished from *B. subtilis*.

Data are presented in graphs with the values for each of the various quality factors indicated in the legend accompanying each figure. Organoleptic grades were determined by the authors in accord with United States Department of Agriculture standards *(15)* and are indicated by the letters on the ordinates of the graphs. Depiction of tryptophan values required off-chart extension at all three temperatures in Figure 3 and at 10° and 20° C. in Figure 4.

RESULTS

The changes which occurred in organoleptic grade, organism population, pH, W.I.A., and tryptophan as a result of inoculating sterile cream with *L. casei* and incubating at various temperatures are shown in Figure 1. Within the range studied, the *L. casei* population increased more rapidly as the temperature increased. Near-maximum populations continued throughout the incubation period, except in the 10° C. sample which exhibited a marked decrease after the seventh day. The development of a high acid flavor caused organoleptic quality to decline in response to increase in cell population and decrease in pH. The W.I.A. fluctuations at each of the three temperatures were beyond the range of experimental error and suggest a slight degree of fat hydrolysis and subsequent fatty acid destruction.

The irregular tryptophan curves followed the same pattern at all three temperatures, which seems unusual when the differences in organism populations

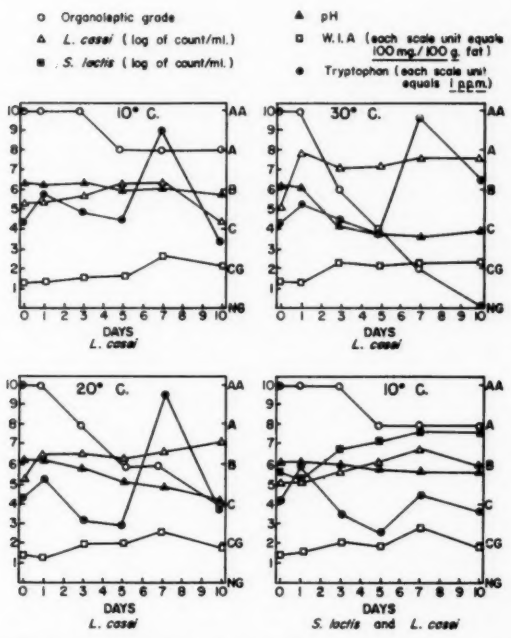

FIG. 1. Organoleptic grade, organism population, pH, W. I. A., and tryptophan values in cream containing *L. casei* or *S. lactis* and *L. casei* as indicated.

are considered. The tryptophan values of sterilized uninoculated controls remained within the range of experimental error throughout the incubation period.

The changes that occurred in organoleptic grade, organism populations, pH, W.I.A., and tryptophan when sterile cream inoculated with *S. lactis* and *L. casei* was incubated at various temperatures are shown in Figures 1 and 2. At each of the three temperatures the population curve of *L. casei* grown with *S. lactis* was similar to that obtained when *L. casei* was grown alone, including a decline in count after the seventh day in the sample held at 10° C. Apparently the presence of *S. lactis* had no influence on *L. casei* population. A comparison of Figures 1 and 2 with Figure 5 indicates that the presence of *L. casei* was slightly inhibitory to the rate of increase and maximum population attained by *S. lactis*. Especially significant is the rapid decrease in *S. lactis* population in the 30° C. sample, with the 10-day count showing a population of only 20 per milliliter.

At 10° C. there was only a slight decline in pH. At 20° C. the pH reduction was greater than that accomplished by either organism individually and at 30° C. the pH values correspond to those produced by *L. casei* alone.

When the two organisms were combined, the slight decrease in W.I.A., which was observed consistently by Harmon and Nelson *(10)* when *S. lactis* was present, occurred on the first day at 20° and 30° C. but was deferred until the

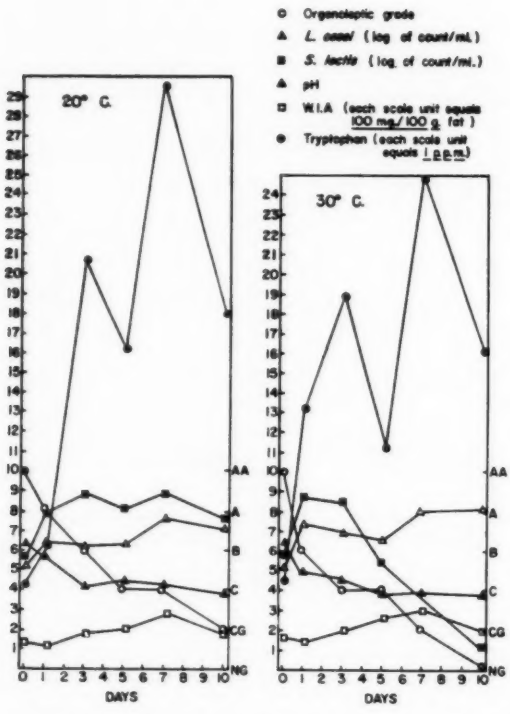

FIG. 2. Organoleptic grade, organism population, pH, W. I. A., and tryptophan values in cream containing *S. lactis* and *L. casei*.

fifth day in the 10° C. sample. At all three temperatures, maximum W.I.A. values occurred on the seventh day but were insufficient to materially influence quality. Decreases between the seventh and tenth days occurred at all temperatures.

At 10° C. the combined organisms caused a fluctuation in tryptophan which was similar to the tryptophan values produced by *L. casei* alone, except that the 7-day value was much lower. At 20° and 30° C. the two organisms together produced extremely high tryptophan values with a saw-tooth curve prevailing after the third day. After the first day, the fact that subsequent tryptophan values were all lower at 30° than at 20° C., even though the same pattern prevailed, may have been due to partial inhibition of the protease by more rapid decrease in pH at 30° C.

Good quality as judged organoleptically was retained throughout the incubation period at 10° C. Rapid quality decline at 20° and 30° C. seemed to be in response to decline in pH and increase in organism population, with high acidity being the principal criticism.

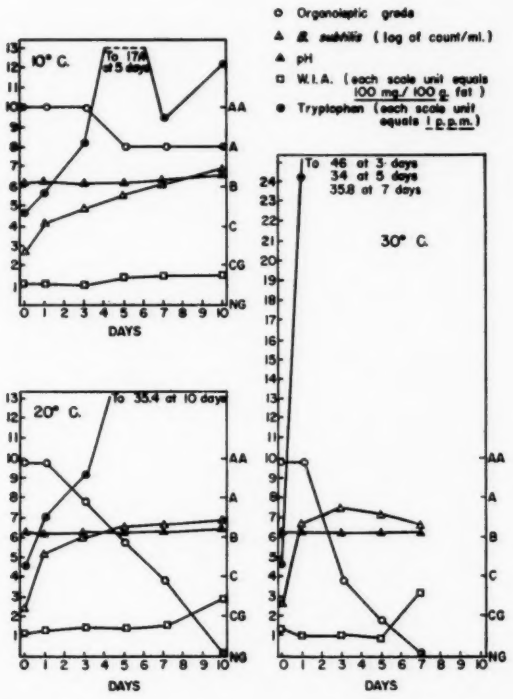

FIG. 3. Organoleptic grade, organism population, pH, W. I. A., and tryptophan values in cream containing *B. subtilis*.

The changes that occurred in organoleptic grade, organism population, pH, W.I.A., and tryptophan in cream inoculated with *B. subtilis* and incubated at various temperatures are shown in Figure 3. The rate of organism population increase and maximum total count attained was greater as the temperature increased within the range studied. At 10° and 20° C. the *B. subtilis* population increased throughout the holding interval, but at 30° C. there was a slight decline after the third day.

There was no significant change in pH at any temperature during the incubation period. A noticeable increase in W.I.A. occurred after the fifth and seventh days at 30° and 20° C., respectively, but the increase was not sufficient to produce a recognizably rancid flavor.

The rapid increase in tryptophan and the high maximum values attained indicate the extremely high degree of proteolysis caused by *B. subtilis*. The amount of proteolysis increased as the temperature and organism population increased, with the rate of increase and maximum population attained being much greater at 30° C. In the sample held at 20° C. the increase in tryptophan was continuous throughout the incubation period, whereas at 10° and 30° C.

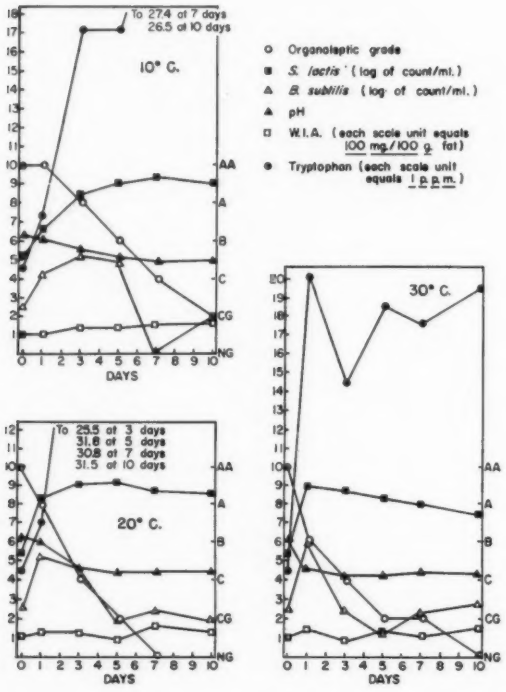

FIG. 4. Organoleptic grade, organism population, pH, W. I. A., and tryptophan values in cream containing *S. lactis* and *B. subtilis*.

maximum tryptophan values were reached at 5 and 3 days, respectively, and were followed by substantial decreases. There was no significant fluctuation in the tryptophan content of an uninoculated control sample. Intensity of the bitter flavor causing deterioration in organoleptic quality seemed to be associated with increase in organism population and proteolysis.

The changes that occurred in organoleptic grade, organism population, pH, W.I.A., and tryptophan when sterile cream was inoculated with both *B. subtilis* and *S. lactis* are shown in Figure 4. When the two organisms were combined, the population curve for *S. lactis* was normal at all temperatures, except that the rate of decline after attaining maximum population was less than that sometimes encountered. The presence of *S. lactis* appeared to become inhibitory to *B. subtilis* at about the time the maximum *S. lactis* population occurred. Also, the maximum rate of decrease in *B. subtilis* lagged behind the greatest decline in pH. Extreme decline in *B. subtilis* population occurred after the first, third, and fifth days at 30°, 20°, and 10° C., respectively. At all temperatures the population decreases were followed by increases that probably were due to the growth of surviving resistant cells of *B. subtilis*.

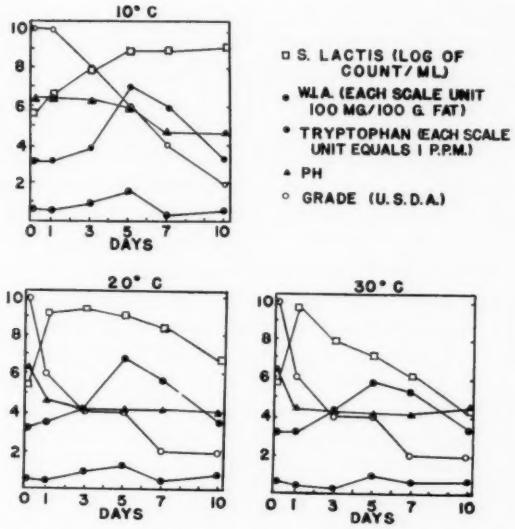

FIG. 5. Organoleptic grade, organism population, pH, W. I. A., and tryptophan values in cream containing *S. lactis*.

Only slight fluctuations occurred in W.I.A. values and these were of no consequence from the organoleptic standpoint. The changes in pH appeared to be in response to *S. lactis* development.

At 10° C. the tryptophan produced by the two organisms was greater than the sum of their individual tryptophan production. At 20° C. the rate of increase was more rapid, but the maximum values attained were slightly less with both organisms present, than with *B. subtilis* alone. At 30° C. the presence of *S. lactis*, which reduced the pH to 4.62 and 4.31 on the first and third days, respectively, materially inhibited the proteolytic ability of *B. subtilis*. Acid and bitter flavors appeared in samples incubated at all three temperatures, with bitterness prevailing at 10° C. and sourness dominating at 30° C. The 20° C. sample was both sour and bitter. In cream containing both organisms, organoleptic quality deterioration was more rapid at 20° than at 30° C., probably owing to the higher pH and greater proteolysis at 20° C.

DISCUSSION

The increases that ocurred in tryptophan when *B. subtilis* and *L. casei* were grown individually and in combination with *S. lactis* in sterile cream were probably due to protein hydrolysis by proteases elaborated by the organisms. It is improbable that any tryptophan was liberated by acid hydrolysis of protein. Hammer and Patil *(9)* and van der Zant *(19)* have reported that 1% lactic acid added to milk did not cause any increase in free tyrosine or tryptophan.

The frequent decreases in tryptophan, encountered when *L. casei* was present, are difficult to explain. Decomposition of tryptophan at low pH resulting from the activity of *L. casei* and *S. lactis* represents a possible cause of reduction which was not investigated. However, there was no variation in tryptophan content when determinations were performed on the extract adjusted to pH intervals of 0.2 between pH 5.0 and 6.0. The possibility also exists that alternate synthesis and hydrolysis of the milk protein may have occurred, as reported by Braz and Allen *(6)*. Decreases would result if some of the tryptophan in peptide form and in position to react with the color reagent used in measuring tryptophan were recombined into larger aggregates.

There are two possible explanations for the uniformity of the tryptophan curves at 10°, 20°, and 30° C., despite the extreme differences in the *L. casei* populations indicated in Figure 1. The optimum temperature for the elaboration of protease by *L. casei* may have been lower than the optimum temperature for cell production, as in the study of *Pseudomonas fragi* reported by Nashif and Nelson *(14)*. Also, the lower pH values resulting from greater acid formation by *L. casei* at the higher temperatures probably reduced the activity of the protease. However, the proteases elaborated from the combination of *S. lactis* and *L. casei* displayed considerable acid tolerance. Increases in tryptophan continued, but at a decreased rate, after the reaction declined below pH 4.5.

When *S. lactis* and *L. casei* were combined, the rapid decrease in *S. lactis* population after the third day in the 30° C. sample probably was caused by the low pH, which was lower than that which occurred when either organism was grown alone. At 20° and 30° C. the combination of organisms gave tryptophan values that were greater than the sum of the quantities produced by the organisms individually, suggesting synergism. Except for greater acid production, the combination of *S. lactis* with *L. casei* did not influence the rate of organoleptic deterioration.

The inhibitory effect of *S. lactis* on *L. casei* populations reported by Baribo and Foster *(2, 3)* was not observed with the combination of organisms used in this work.

Within the range studied, both the population of *B. subtilis* and the activity of the protease elaborated therefrom increased as the temperature increased. When *S. lactis* and *B. subtilis* were combined, a decline in rate of proteolysis and *B. subtilis* population occurred after the pH decreased to about 5.5. Proteolysis ceased when the pH reached 4.50 to 4.55. The *B. subtilis* count ultimately declined to less than 500 per milliliter at all incubation temperatures, with the rate of decrease being related to pH decline.

It is noteworthy that increases in tryptophan continued after the population of both organisms began to decline. There are several possible explanations. Even in a declining population new cells may continue to be produced and liberate protease. Protease may have been liberated from dead cells and also the autolysis of dead cells may have contributed directly to the tryptophan content. Marshall *(13)* and Cox and Whitehead *(7)* reported that *B. subtilis* in small numbers stimulated the population and acid production of *S. lactis*.

No stimulating influence was evident in this work, in which the initial inoculation averaged about 250,000 *S. lactis* and 500 *B. subtilis* organisms per milliliter. The addition of *S. lactis* to *B. subtilis* increased the rate of proteolysis and organoleptic deterioration at 10° and 20° C., whereas at 30° C. the proteolysis was retarded and rate of organoleptic deterioration was not materially influenced.

Bitter flavors suggestive of proteolysis were uniformly present in cream containing 18 to 25 or more p.p.m. of tryptophan. Combining *S. lactis* with *B. subtilis* and with *L. casei* did alter the tryptophan level at which bitter or putrid flavors were apparent.

Among the entire group of inoculated samples held at various temperatures, *B. subtilis* in pure culture at 20° C. was the only one in which a tryptophan value in excess of 10 p.p.m. was associated with a sample of cream scoring above 90 or grade B.

SUMMARY

1. When sterile cream was inoculated with *Lactobacillus casei* and incubated at 10°, 20°, and 30° C., the maximum tryptophan was 9.4 p.p.m. and occurred in the 30° C. sample after 7 days incubation.

2. When *Streptococcus lactis* and *L. casei* were inoculated into sterile cream, *S. lactis* had no influence on *L. casei* population. *L. casei* slightly inhibited the maximum *S. lactis* population attained, and after 10 days at 30° C. the *S. lactis* count decreased to 20 per milliliter. The maximum tryptophan produced by the combination of *S. lactis* and *L. casei* was 29.6 p.p.m. and occurred in the 20° C. sample after 7 days incubation.

3. When *Bacillus subtilis* was inoculated into sterile cream, the organism population and amount of proteolysis increased as the temperature increased, with a maximum tryptophan value of 46 p.p.m. occurring in the 30° C. sample after incubation for 3 days.

4. When *S. lactis* and *B. subtilis* were inoculated into sterile cream, substantial proteolysis occurred at all temperatures. The *B. subtilis* population declined rapidly after the maximum *S. lactis* population was attained.

5. Bitter flavors suggestive of proteolysis were uniformly present in cream containing 18 to 25 or more p.p.m. of tryptophan. Tryptophan values of 10 p.p.m. or more were usually associated with cream scoring 90 (grade B) or lower, but all cream of this quality did not possess high tryptophan values.

6. None of the organisms or combinations used in this work produced W.I.A. values high enough to be significant from the quality standpoint.

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INTERRELATIONSHIPS OF MICROORGANISMS IN CREAM. III. *CANDIDA PSEUDOTROPICALIS, AEROBACTER AEROGENES,* AND *STREPTOCOCCUS LACTIS*[1]

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Candida pseudotropicalis, and Aerobacter aerogenes were inoculated individually and in combination with Streptococcus lactis into sterile cream which was incubated at different temperatures and analyzed for protein and fat decomposition, pH, growth and flavor changes. *Editor.*

Regulatory agencies and the dairy industry have been interested in determining the amounts of proteolysis, lipolysis and various other deterioration values in cream and butter. These may result from the growth of common contaminating organisms. Previous reports by Harmon and Nelson *(7, 8)* have dealt with changes in water-insoluble acids (W.I.A.) and tryptophan values produced when certain common lipolytic or proteolytic organisms were inoculated into sterile cream.

In the work reported herein we determined the effect of representative gas-producing organisms on W.I.A. and tryptophan values in cream. Samples of sterile cream inoculated with *Candida pseudotropicalis (Torula cremoris)* and *Aerobacter aerogenes,* individually and in combination with *Streptococcus lactis,* deteriorated rapidly as determined organoleptically, but deterioration measured by chemical methods was less apparent.

Very little work has been reported in measuring the proteolytic and lipolytic ability of coliform bacteria and yeasts. Berger *et al. (2)* found moderate peptidase activity by cell-free extracts obtained from freezing and thawing cultures of *Escherichia coli,* with the maximum peptide hydrolysis occurring at pH 8.0 to 9.0. The inhibitory effect of rancid milk on certain bacteria was studied by Costilow and Speck *(4).* Milk that had undergone lipolysis to the extent that the surface tension was reduced about 10 dynes per centimeter was found to have a slightly inhibitory effect on *E. coli.* Cox and Whitehead *(5)* grew *E. coli* in association with lactic streptococci and found that the acidity produced was variable, with *E. coli* causing an increase in acid production in some experiments and a decrease in others. Collins and Nelson *(3)* found that when *E. coli* and *A. aerogenes* were grown alone in milk at 30° C. they caused a deficit in soluble nitrogen during the first few days, which was later overcome, and marked increases in soluble nitrogen occurred between 7 and 15 days. Mixed

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cultures of *S. lactis* and coliform organisms caused gradual increases in soluble nitrogen, but the values at 15 days never were as high as those for the coliform organisms alone and were about the same magnitude as those for *S. lactis*.

EXPERIMENTAL PROCEDURE

Proteolysis, lipolysis, organism population, pH, and organoleptic grade resulting from the growth of *C. pseudotropicalis* and *A. aerogenes*, individually and in combination with *S. lactis*, were determined in sterile cream incubated at various temperatures. Cream samples were prepared, inoculated, incubated, sampled, and analyzed according to procedures outlined previously by Harmon and Nelson *(7)*. Organoleptic grades were determined by the authors in accord with United States Department of Agriculture standards *(10)*. The letters on the right ordinate of the graphs represent the appropriate U.S.D.A. grades.

Lipolysis was measured by the Hillig *(9)* method for determining W.I.A., and proteolysis was measured by the Duggan *(6)* method for determining free tryptophan. Plate counts for organisms were performed by Standard Methods *(1)* procedures. When *S. lactis* and *A. aerogenes* were grown as mixed cultures, it was not always possible to differentiate between the colonies; therefore, *S. lactis* populations were determined by subtracting the coliform counts obtained on desoxycholate agar from the total populations appearing on tryptone glucose extract milk (T.G.E.M.) agar. On T.G.E.M. agar plates incubated at 32° C. for 48 hours there was sufficient difference in colony appearance to distinguish *S. lactis* from *C. pseudotropicalis*.

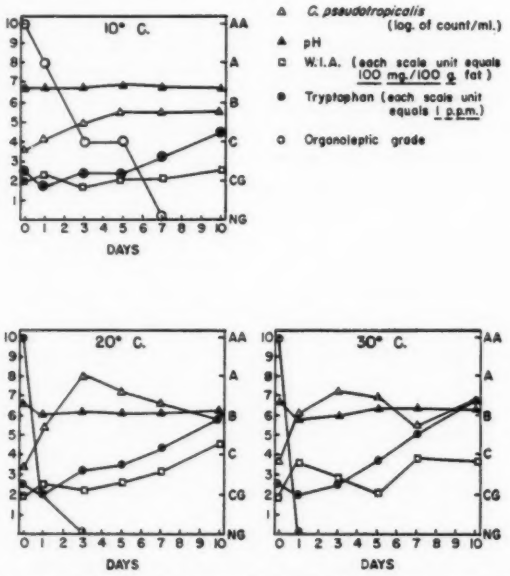

FIG. 1. W. I. A., tryptophan, pH, organoleptic grade, and organism population of cream containing *C. pseudotropicalis*.

RESULTS

The changes which occurred in W.I.A., tryptophan, pH, organoleptic grade, and organism population when sterile cream was inoculated with *C. pseudotropicalis* and incubated at 10°, 20°, and 30° C. are shown in Figure 1. At 10° C. the organism population increased slowly, attained maximum on the fifth day, and then became stationary. At 20° and 30° C. the population increased more rapidly, reached maximum on the third day, and gradually declined; however, at 30° C. there was a secondary increase after the seventh day. At all temperatures the W.I.A. values showed a general tendency to increase, although there was a uniform slight decline on the third day. The tryptophan values decreased uniformly on the first day at all temperatures and then gradually increased throughout the remainder of the incubation period. The organoleptic deterioration was so rapid that no W.I.A. or tryptophan fluctuations of consequence occurred during the commercial life. Fluctuations in pH were attributed to differences in the retention of gas formed during fermentation.

Figure 2 illustrates the changes that occurred in W.I.A., tryptophan, pH, organoleptic grade, and organism population when sterile cream was inoculated with both *C. pseudotropicalis* and *S. lactis* and incubated at various temperatures. A comparison of Figures 1 and 2 indicates that the presence of *S. lactis* diminished the maximum population attained by *C. pseudotropicalis*, with the reduction being greater at 20° C. than at 10° and 30° C. When *S. lactis* was

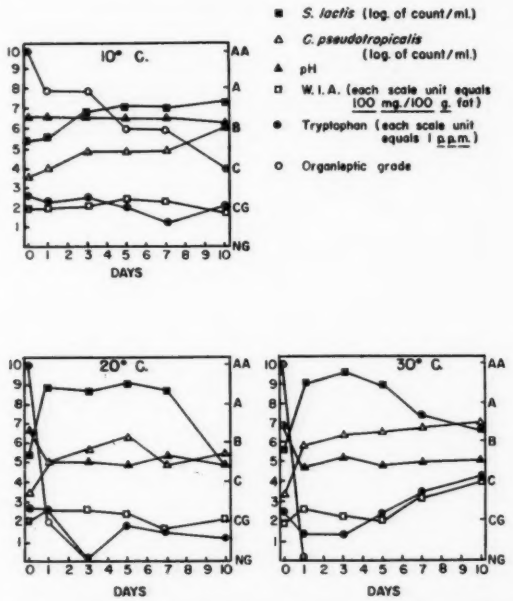

FIG. 2. W. I. A., tryptophan, pH, organoleptic grade, and organism population of cream containing C. *pseudotropicalis* and *S. lactis*.

present, a lag in the growth rate of *C. pseudotropicalis* occurred at all temperatures. The pH did not drop as low as normal for a culture containing *S. lactis*, suggesting that *C. pseudotropicalis* may have either interfered with the acid-producing ability of *S. lactis* or utilized some of the acid. A comparison of the W.I.A. curves in Figures 1 and 2 and Figure 5 of the preceding article *(8)* shows that the tendency of *C. pseudotropicalis* to cause a slight early increase in W.I.A. is more dominant than the ability of *S. lactis* to cause a decrease during the corresponding period. The early slight decrease in tryptophan caused by *C. pseudotropicalis* also occurred when the yeast was grown in association with *S. lactis*. W.I.A. and tryptophan values did not reach significant levels at any of the three temperatures during the commercial life of the cream. At 20° and 30° C., addition of *S. lactis* did not alter the rate of organoleptically determined deterioration caused by *C. pseudotropicalis*. However, at 10° C., *S. lactis* exhibited a protective effect on the quality of the cream, and the population attained by *C. pseudotropicalis* in the presence of *S. lactis* was lower than when *C. pseudotropicalis* was grown alone. At 10° C. the cream containing only *C. pseudotropicalis* had a much more intense yeast flavor and was more foamy than the cream containing both *C. pseudotropicalis* and *S. lactis;* the rate of organoleptic deterioration of the former was much more rapid.

The changes that occurred in W.I.A., tryptophan, pH, organoleptic grade, and organism population when sterile cream was inoculated with *A. aerogenes* and incubated at various temperatures are shown in Figure 3. At 10° C. the organism population decreased throughout the incubation period and no signifi-

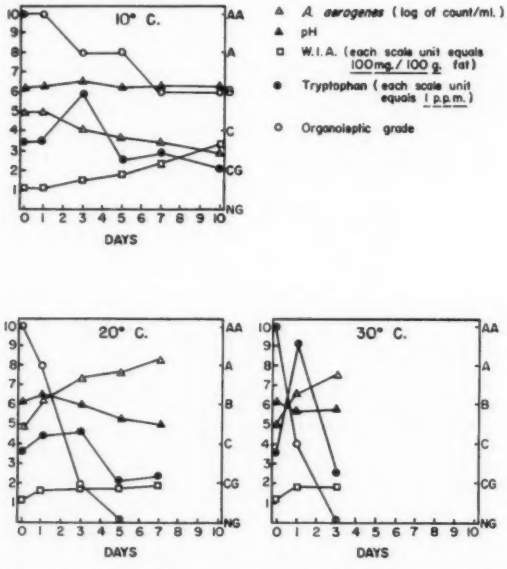

FIG. 3. W. I. A., tryptophan, pH, organoleptic grade, and organism population of cream containing *A. aerogenes*.

cant change occurred in pH. At 20° and 30° C. the counts increased and the pH decreased, with more rapid deterioration occurring at 30° C. In the 30° C. sample, gas production was so great that the cream "foamed over" after about 40 hours. Very slight gradual increases occurred in W.I.A. at all temperatures. However, at 30° C. the commercial life of the sample was too short to determine a trend. At all temperatures the tryptophan values maintained a uniform trend of an early increase followed by a decrease, although the increase at 20° C. was only slight. The decline in quality measured organoleptically seemed to be more closely related to organism population than to any other factor considered. From the standpoint of physical effect on the cream and intensity of off-flavor produced at 20° and 30° C., *A. aerogenes* was an extremely objectionable organism because of the foamy body and unclean flavor. The degree of intensity of off-flavor was not associated with W.I.A. or tryptophan values.

Figure 4 illustrates the changes which occurred in W.I.A., tryptophan, pH, organoleptic grade, and organism population when sterile cream was inoculated with both *A. aerogenes* and *S. lactis* and incubated at 10°, 20°, and 30° C. At 10° C. the count on the coliform organism decreased from an initial 74,000 to 38 per gram on the tenth day. Despite the rapid decline in population, *A. aerogenes* exhibited a definite inhibitory effect on *S. lactis*, evidenced by the fact that the rate of increase in *S. lactis* population was much slower than normal. At 10° C. there was no apparent change in pH, a further indication that *S. lactis* was in-

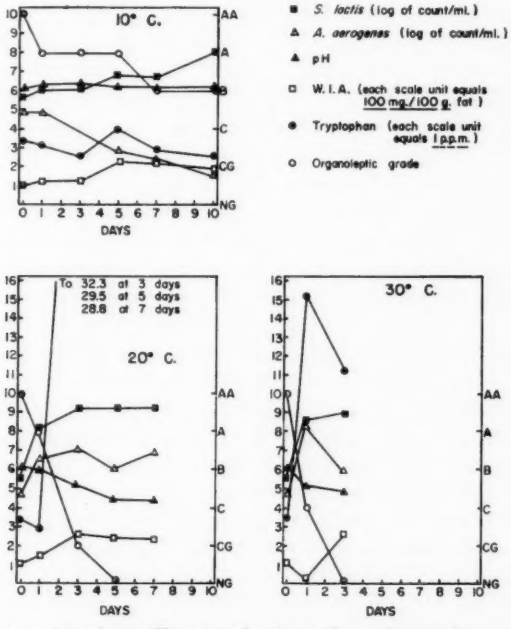

FIG. 4. W. I. A., tryptophan, pH, organoleptic grade, and organism population of cream containing *A. aerogenes* and *S. lactis*.

hibited. At 20° C. the *S. lactis* increase was normal and a comparison of Figures 3 and 4 indicates that *S. lactis* inhibited the coliform population to some extent. The sample held at 30° C. "foamed over" after about 40 hours of incubation. A portion was salvaged, refrigerated, and analyzed on the third day. The drop in *A. aerogenes* population in this sample was probably due to antagonism from *S. lactis,* since the corresponding sample containing only *A. aerogenes* endured the same handling and showed an increase in population on the third day. No change of any consequence occurred in the W.I.A. values at either of the three temperatures, although at 30° C. there was an early decrease, probably attributable to *S. lactis.* Combination of the two organisms produced marked increases in tryptophan at 20° and 30° C., with the 20° C. sample reaching an extremely high value of 32 p.p.m. on the third day. The tryptophan values were much greater than either organism was able to produce singly, and in the 20° C. sample the value was twice as much as the cumulative effect of the two organisms functioning independently. Organoleptically detectable deterioration was severe at the two higher temperatures, with a sour, unclean flavor and gassy body becoming evident early in the incubation period. At 10° C. the keeping quality was fairly good, with *A. aerogenes* seeming to survive long enough to interfere with normal acid development by *S. lactis.*

DISCUSSION

Neither *C. pseudotropicalis* nor *A. aerogenes* inoculated into sterile cream, individually and in combination with *S. lactis,* and incubated at 10°, 20°, and 30° C. caused lipolysis exceeding 400 mg. of W.I.A. per 100 g. of fat during the commercial life of the cream. Increases and fluctuations which occurred in W.I.A. values were moderate, with slight increases being attributed to fat hydrolysis by the organism and decreases being attributed to destruction or utilization of long chain fatty acids.

Maximum standards for free tryptophan have not been established. However, Harmon and Nelson *(8)* reported that bitter flavors, suggestive of proteolysis, usually were present in cream containing 18 to 25 p.p.m. of free tryptophan and cream or butter containing 10 p.p.m. usually graded B or lower. *C. pseudotropicalis,* individually and in combination with *S. lactis,* did not cause any increase in tryptophan at any of the incubation temperatures during the commercial life of the cream. Considerable fluctuation occurred in the tryptophan content of cream inoculated with *A. aerogenes.* These fluctuations may be due to autolysis of cells or to a lack of balance between tryptophan liberation by the protease and utilization by the organism. When *S. lactis* and *A. aerogenes* were combined at 10° C., this temperature was probably too low to permit the development of sufficient population to produce proteolysis. The extremely high tryptophan values which occurred at 20° C. seemed to result from synergistic action of the two organisms, since the quantity greatly exceeded the combined tryptophan production of the two organisms when grown individually [Figure 3 and Figure 5 of the preceding paper *(8)*]. The interruption in protease activity in the 30° C. sample, after the first day, was prob-

ably due to the rapid decline in pH. At 20° C. the rate of decline in pH was slower and vigorous protease activity continued until the third day before leveling off or decreasing. No increases in tryptophan occurred after the pH dropped to 5.2. Also, the maximum tryptophan values were coincident with the attainment of maximum organism population and stabilized or slightly decreased as the organism population leveled off or declined.

When *S. lactis* was combined with *C. pseudotropicalis* in sterile cream, some inhibition of the cell population of *C. pseudotropicalis* was apparent at all temperatures, and at 10° C. the commercial life of the cream was longer than that of a companion sample containing only *C. pseudotropicalis*. Although the yeasts are considered to be acid tolerant, the most logical reason for reduction of *C. pseudotropicalis* population seemed to be the low pH caused by *S. lactis* action. This assumption is supported by the fact that plate counts of *C. pseudotropicalis* were substantially lower on acidulated potato dextrose agar than on T.G.E.M. agar. *S. lactis* provided a competitive factor in addition to low pH, since the *C. pseudotropicalis* population was lower in the 10° C. sample in which there was very little decline in pH.

The presence of *C. pseudotropicalis* seemed to alter the normal fermentation of *S. lactis* [Figure 2 and Figure 5 of the preceding paper (8)], in that the acid producing ability was diminished and there was no noticeable inclination for *S. lactis* to utilize W.I.A. or produce free tryptophan.

Low temperature seemed to be the only factor responsible for the abrupt decrease in *A. aerogenes* population at 10° C.; however, many coliform bacteria are capable of increasing at this temperature. Combination of *S. lactis* with *A. aerogenes* did not minimize the rapid rate of organoleptically measurable deterioration, but the acid produced by *S. lactis* caused an abrupt decline in *A. aerogenes* population after the pH dropped to about 5.2.

SUMMARY

1. *Candida pseudotropicalis* and *Aerobacter aerogenes*, individually and in combination with *Streptococcus lactis*, were inoculated into sterile cream and incubated at 10°, 20°, and 30° C. for 10 days or until the cream was no longer of acceptable commercial quality, whichever occurred first.

2. Neither of the organisms, individually or in combination with *S. lactis*, produced as much as 400 mg. of W.I.A. per 100 g. of fat.

3. *C. pseudotropicalis*, alone or in combination with *S. lactis*, and *A. aerogenes* alone caused negligible increases in free tryptophan. The combination of *A. aerogenes* and *S. lactis* produced 32 p.p.m. of free tryptophan in 3 days at 20° C. and 15 p.p.m. in 1 day at 30° C.

4. At 20° C. the combination of *S. lactis* with *C. pseudotropicalis* diminished the rate of increase and maximum *C. pseudotropicalis* population attained. The presence of *A. aerogenes* caused a reduction in the rate of increase and maximum population attained by *S. lactis*.

5. At 20° and 30° C., addition of *S. lactis* did not alter the rate of organoleptically determined deterioration caused by *C. pseudotropicalis*, but at 10° C. the

presence of *S. lactis* extended the commercial life of cream containing *C. pseudotropicalis*. Deterioration in organoleptic quality appeared to be more closely related to increases in organism populations than to chemical factors studied.

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THE REACTIVATION OF PHOSPHATASE IN H.T.S.T. PASTEURIZED DAIRY PRODUCTS

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The phosphatase test has been accepted as infallible for determining if milk products have been properly pasteurized. Recently, however, in this country and abroad, it has been shown that milk and cream may be heated to the proper H.T.S.T. pasteurization and even to sterilization temperatures and reveal positive phosphatase tests after as little as 2 hours storage at 75° to 80° F. This reactivation of the enzyme, phosphatase, does not occur when fluid milk products are stored at 40° F. or lower. *Editor.*

The phosphatase test has long been accepted as a means of determining whether or not a sample of milk or cream has received proper and safe pasteurization treatment. Recently, a positive phosphatase test was found in 20% cream pasteurized by the short time method. The heat treatment used for this product was in excess of accepted safe pasteurization standards (172° F. for 20 seconds), yet the positive tests, according to public health officials, would indicate under-pasteurization.

An investigation of the problem indicated that the cream immediately after heat treatment had a negative phosphatase reaction, but when allowed to stand at room temperature (75° to 80° F.) for as little as 2 hours, a positive phosphatase test was obtained. No change in phosphatase reaction was obtained when the cream was properly refrigerated and, more significantly, the reappearance of the phosphatase did not occur in long time pasteurized cream. Bacteriological examination of the H.T.S.T. pasteurized cream, when the positive phosphatase was found, indicated little or no significant change in bacterial numbers during the 2-hour storage period, making it unlikely that the phosphatase which developed during this time was of bacterial origin. It appeared that true enzyme reactivation was involved. Although this phenomenon has been frequently reported *(2, 7, 8, 12)*, it is believed that this is the first evidence that reactivation of the phosphatase has occurred in a commercially pasteurized product.

This study was undertaken to confirm these findings in the laboratory and to determine more specifically the conditions under which the reactivation of the phosphatase occurred in H.T.S.T. pasteurized cream and other dairy products. This information, it is hoped, will help clarify the role of the phosphatase test for quality control, especially as it applies to H.T.S.T. pasteurized dairy products.

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EXPERIMENTAL PROCEDURES

Skimmilk, whole milk, cream containing 20% and 38% fat were used in this study. The products were pasteurized in a 50-gal. per hour H.T.S.T. tubular heater, with a 16-second holding tube, at 165° to 240° F. Owing to difficulties in heating the heavy cream, this product was heated only at 165° and 185° F. In addition, each product was laboratory pasteurized at 145° to 150° F. for 30 minutes. In an attempt to determine the holding time at a given temperature which would prevent subsequent reactivation, some heavy cream samples were also heated at 165°, 180°, and 195° F. for holding times ranging from 20 seconds to 8 minutes. These treatments were conducted in a water bath, with 2 ml. of cream in a thin-walled vial for each time/temperature treatment, and with constant manual agitation throughout the heating period. A thermocouple inserted in the vial was used to determine when each sample had reached the desired pasteurizing temperature and, after the specified holding time, each vial was rapidly cooled by immersing in an ice water bath.

Each sample, except those processed in the vials, was examined for phosphatase and standard plate count immediately after pasteurization and again after various time intervals at 88° F. storage. Control samples of each product were held at 40° F. and examined for phosphatase, along with the test samples stored at the elevated temperature. The vial samples were examined for phosphatase immediately after heating and again after 2 hours at 88° F. storage. The Scharer Rapid Phosphatase Test, as supplied by Applied Research Institute, was used for all phosphatase determinations. The development of any blue color with this method was considered as a positive test for phosphatase. Although this method is not a quantitative one, it is widely used and, with suitable controls, has been found to be reliably sensitive *(3)*. Boiled controls of each product being tested and raw controls made up with 0.1% raw product were run with each determination. The bacteria counts were made according to procedures described in the 10th Edition of Standard Methods for the Examination of Dairy Products.

In order to differentiate between phosphatase of bacterial origin and true milk phosphatase, all samples that showed reactivated phosphatase were repasteurized at 145° F. for 30 minutes and immediately reexamined for phosphatase. A negative test after this additional heat treatment would indicate reactivated milk phosphatase, since all known bacterial phosphatase has been reported to resist 145° F. for 30 minutes *(1, 12)*.

In addition to these laboratory studies, further observations were made on commercially short-time pasteurized 20% cream. A sample of cream from each day's production (over a period of 15 days) was examined for phosphatase and standard plate count immediately after heat treatment, and again after holding the cream for 2 hours at 86° F., and after 48 to 96 hours at 40° F.

RESULTS

Tables 1 to 4 summarize the phosphatase and standard plate count data obtained for each product. In each case, the phosphatase reaction immediately

after pasteurization (both long- and short-time heated) was negative. When the samples were stored at 88° F., however, only short-time pasteurized products showed positive phosphatase tests. The minimum temperature above which reactivation of the enzyme took place and the length of time at 88° F. required for the development of the positive tests appeared to be directly related to the fat content of the product. In addition, it appeared that more enzyme reactivation was noted in each product as the pasteurization temperature was increased. Skimmilk (Table 1) showed reactivated phosphatase only when heated at 210° F. or above and required approximately 18 hours at 88° F. storage before the positive tests were found. In whole milk (Table 2), the reactivation of the enzyme occurred when the milk was heated at 195° F. or above and was found after only 6 hours time at 88° F. In 20% cream and 38% cream (Tables 3 and 4), the reactivation was found in samples heated only to 180° F. or above and 165° F. or above, respectively, and within only 2 hours at 88° F. In no case did the long-time pasteurized products show evidence of reactivated phosphatase

TABLE 1

Phosphatase and bacterial development in pasteurized skimmilk held 18 hours at 88° F.

Processing treatment	Phosphate reaction			Standard plate count (bacteria/ml)	
	Immediately after pasteurization	After 18 hr. at 88° F.	After repasteurization at 145° F/30 min.	Immediately after pasteurization	After 18 hr. at 88° F.
240° F/16 sec.	−	+	−	16	40,000
225° F/16 sec.	−	+	−	40	12,000
210° F/16 sec.	−	+	−	300	2,000,000
195° F/16 sec.	−	−	−	450	17,000,000
180° F/16 sec.	−	−	−	700	17,000,000
165° F/16 sec.	−	−	−	4,000	700,000,000
145-150° F/30 min.	−	−	−	6,200	42,000,000

Initial raw milk phosphatase +
Initial raw milk bacteria count 770,000/ml.

TABLE 2

Phosphatase and bacterial development in pasteurized whole milk held 6 hours at 88° F.

Processing treatment	Phosphatase reaction			Standard plate count (bacteria/ml.)	
	Immediately after pasteurization	After 6 hr. at 88° F.	After repasteurization at 145° F/30 min.	Immediately after pasteurization	After 6 hr. at 88° F.
240° F/16 sec.	−	+	−	10	140
225° F/16 sec.	−	+	−	140	110
210° F/16 sec.	−	+	−	150	290
195° F/16 sec.	−	+	−	300	250
180° F/16 sec.	−	−	−	1,100	3,300
165° F/16 sec.	−	−	−	8,000	14,600
145-150° F/30 min.	−	−	−	4,100	4,500

Initial raw milk phosphatase +
Initial raw milk bacteria count 960,000/ml.

TABLE 3

Phosphatase and bacterial development in pasteurized 20% fat cream held 2 hours at 88° F.

Processing treatment	Phosphatase reaction			Standard plate count (bacteria/ml.)	
	Immediately after pasteurization	After 2 hr. at 88° F.	After repasteurization at 145° F/30 min.	Immediately after pasteurization	After 2 hr. at 88° F.
240° F/16 sec.	−	+	−	10	5
225° F/16 sec.	−	+	−	11	16
210° F/16 sec.	−	+	−	200	220
195° F/16 sec.	−	+	−	300	270
180° F/16 sec.	−	+	−	400	370
165° F/16 sec.	−	−	−	3,500	4,100
145-150° F/30 min.	−	−	−	4,500	7,000

Initial ray cream phosphatase +
Initial raw cream bacteria count 500,000/ml.

TABLE 4

Phosphatase and bacterial development in pasteurized 38% fat cream held 2 hours at 88° F.

Processing treatment	Phosphatase reaction			Standard plate count (bacteria/ml.)	
	Immediately after pasteurization	After 2 hr. at 88° F.	After repasteurization at 145° F/30 min.	Immediately after pasteurization	After 2 hr. at 88° F.
185° F/16 sec.	−	+	−	180	300
165° F/16 sec.	−	+	−	2,100	3,700
145-150° F/30 min.	−	−	−	7,800	8,900

Initial raw cream phosphatase +
Initial raw cream bacteria count 116,000/ml.

when held at the elevated temperature. Also, with each product, all the control samples (both long- and short-time pasteurized) when held at 40° F., continued to show negative phosphatase even after 7 days storage. At any time during this storage at 40° F., the reactivation of the enzyme could be demonstrated in the H.T.S.T. products that initially showed reactivation, by simply holding those samples under the conditions described above.

The effect of increased holding time on the reactivation of the phosphatase in heavy cream, at temperatures which previously had produced reactivation when the cream was pasteurized for 16 seconds, is summarized in Table 5. At each time/temperature interval studied, initial phosphatase reaction was nega-

TABLE 5

The effect of holding time on the reactivation of phosphatase in 38% fat cream pasteurized at three different temperatures

Pasteurization temperature	Phosphatase reaction in samples after holding 2 hr. at 88° F. Processed at various times					
	20 sec.	*40 sec.*	*60 sec.*	*2 min.*	*4 min.*	*6 min.*
165° F.	+	−	−	−	−	−
180° F.	+	+	+	+	+	−
190° F.	+	+	+	+	−	−

tive immediately after heating. When the samples were subequently held for 2 hours at 88° F., the intensity of the reactivated phosphatase decreased as the holding time was increased. These results would indicate that holding time is more critical than the pasteurizing temperature in preventing the reactivation of the phosphatase.

Bacteriologically, there was essentially no increase in the numbers of organisms in the milk, 20% cream, or 38% cream at the end of the storage period at 88° F. when the positive phosphatase test in each product was observed (Tables 2, 3, and 4). These results would indicate conclusively that the reappearance of the phosphatase enzyme in dairy products following H.T.S.T. treatment was not due to phosphatase producing bacteria growing in the products. In the skimmilk (Table 1), the length of time required at 88° F. before the reactivation of the enzyme was observed was 18 hours and, during this time, considerable increase in bacterial numbers had taken place. However, considerably more bacterial growth had taken place in the samples that had received the lower heat treatments and had not shown the phosphatase reactivation. Also, when each sample that had shown enzyme reactivation was repasteurized at 145° F. for 30 minutes, complete destruction of the reactivated phosphatase resulted, again indicating that the positive tests were not of bacterial origin since bacterial phosphatase is reportedly more heat-resistant than milk phosphatase and will survive exposure to 145° F. for 30 minutes.

To demonstrate further that the reactivated phosphatase was not of bacterial origin, a number of organisms were isolated from the skimmilk samples which showed positive phosphatase after the 18-hour storage time at 88° F. and were inoculated into sterile milk and cream. Although many of the isolants were active phosphatase producers, positive tests could not be found in the inoculated samples after 18 to 24 hours, in spite of counts in the millions per milliliter. Phosphatase activity of the isolants was determined on a water suspension of the culture made by suspending the growth from an agar slant in 10 ml. sterile distilled water. The increased heat resistance of bacterial phosphatase was also confirmed by subjecting water suspensions of the phosphatase-producing bacteria to pasteurizing treatment of 145° F. for 30 minutes and demonstrating no loss in enzyme activity.

Since the reactivation of the phosphatase enzyme in the H.T.S.T. products appeared to be a function of the pasteurizing temperature (the higher the temperature, the more likely and greater the reactivation), it was thought that the positive tests might simply be due to a heat induced reaction. To determine if this was so, a sample of 20% cream was first pasteurized at 145° F. for 30 minutes (it has been shown that phosphatase in the cream will not reactivate after this heating), then short-time heated for 16 seconds at temperatures up to 200° F. Subsequent storage of the cream samples for as long as 24 hours at 88° F. indicated no reactivation in any of the samples. Apparently, the long-time treatment completely and permanently denatured the phosphatase enzyme in the cream, so that subsequent exposure to the H.T.S.T. treatment had no further effect on the enzyme.

TABLE 6

Phosphatase and bacterial development in commercially short-time pasteurized 20% cream held at 75-80° F. and at 40° F.

Date	Phosphatase reaction			Standard plate count (bacteria/ml.)		
	Immediately after pasteurization	After 2 hr. at room temp.	After 48-96 hr. at 40° F.	Immediately after pasteurization	After 2 hr. at room temp.	After 48-96 hr. at 40° F.
9/19	−	+	−	2,100	2,400	2,300
9/20	−	+	−	3,100	3,500	3,600
9/21	−	+	−	2,200	2,300	6,800
9/22	−	+	−	4,000	18,000	37,000
9/23	−	−	−	1,600	3,700	3,900
9/26	−	+	−	1,900	2,300	3,200
9/27	−	+	−	2,400	4,200	3,000
9/28	−	+	−	4,500	4,600	2,800
9/29	−	+	−	1,800	2,400	3,800
9/30	−	+	−	3,200	4,100	4,200
10/3	−	−	−	800	1,700	1,400
10/4	−	+	−	3,000	3,100	1,600
10/5	−	+	−	2,000	4,000	3,100
10/6	−	+	Not done	1,400	12,000	Not done
10/7	−	+	Not done	4,300	7,200	Not done

A summary of the data obtained on the commercially short-time pasteurized cream is presented in Table 6. The cream at this plant was pasteurized in a conventional plate heater at 172° F. for approximately 20 seconds. On each of the 15 days when samples were examined, negative phosphatase tests were obtained on all samples immediately after pasteurization. When these same samples were held for 2 hours at 75° to 80° F., 13 out of 15 samples tested showed positive phosphatase but, when held for as long as 96 hours at 40° F., all 15 samples remained negative. The data again indicated no significant change in numbers of bacteria during storage, confirming the earlier finding that true enzyme reactivation occurred in commercially H.T.S.T. pasteurized cream.

DISCUSSION

The results of this study confirm the many reports in the literature *(2, 8, 10, 11, 12)* that true reactivation of milk phosphatase occurs in H.T.S.T. pasteurized dairy products but does not occur in similar products pasteurized by the long-hold method. Since it appears that more and more fluid dairy products will be processed by short-time methods, and with greater emphasis in the future most likely to be on higher temperatures for shorter times, it would appear that a reevaluation of the phosphatase test as it applies to H.T.S.T. products is in order.

This is especially true of cream, which appears to be particularly susceptible to phosphatase reactivation after H.T.S.T. treatment. As was demonstrated with the commercially processed H.T.S.T. cream samples, a sample of 20% cream, inadvertently left at room temperature for as little as 2 hours before testing, might show a positive phosphatase test and a faulty interpretation of pasteurization efficiency. Heavy cream pasteurized under similar H.T.S.T.

conditions also would require careful phosphatase interpretation, since it shows enzyme reactivation after even lower pasteurizing temperatures than light cream. Fluid whole milk and skimmilk, on the other hand, do not show reactivation of the phosphatase unless heated at temperatures considerably higher than the 162° F. for 16 seconds now used, and consequently it is unlikely that positive tests (reactivation) would be found in these products when commercially pasteurized.

Some preliminary observations in this laboratory with H.T.S.T. sterilized milk have shown that milk heated to 260° to 280° F. for several seconds, designed for nonrefrigerated storage, also shows reactivated phosphatase within 24 hours after processing.

If the phosphatase test is to be of value in determining if milk and cream have received proper H.T.S.T. pasteurization, it is essential to make phosphatase tests immediately after heating. Otherwise, it is necessary to insure that the products have never been above 40° to 50° F.

Since the reactivation of phosphatase has occurred more frequently in cream than in milk, many of the attempts to explain the cause of the phenomenon have been based on the fact that the higher concentration of the enzyme in the cream does not follow the same heat destruction pattern as in milk. The suggestion has been made *(7, 9)* that a small portion of the phosphatase in cream is "bound" by the fat in such a way that it escapes destruction during the very short heat treatment. Immediately after heat treatment, a negative test is obtained but, after a short storage period, the undestroyed phosphatase diffuses into the serum and a positive test is obtained. This hypothesis, if true, would hardly explain the results reported in this paper, which indicated that more phosphatase reactivation was obtained as the processing temperature was increased.

There have also been several reports *(1, 5, 6)* suggesting that the reappearance of phosphatase after pasteurization is due to bacterial growth during storage. However, these studies indicated that in spite of the fact that the production of bacterial phosphatase is common to many organisms normally found in dairy products, considerable growth of these organisms (resulting in counts in the millions per milliliter) is necessary before a positive phosphatase test is obtained. The evidence reported in this paper and the fact that the reactivation of phosphatase has been observed in milks subjected to short time sterilizing temperatures *(4, 10)* would most certainly rule out bacterial phosphatase as a factor in the phenomenon.

No attempt has been made in this study to investigate the mechanism of phosphatase reactivation, or to characterize and compare the reactivated milk phosphatase with the original milk phosphatase. These and other fundamental aspects of the reactivation phenomenon have been published by Wright and Tramer *(10, 11, 12, 13)*.

SUMMARY AND CONCLUSIONS

1. The cause of positive phosphatase tests in commercially short-time pasteurized cream was found to be reactivation of the enzyme, which occurred

when the cream was held for as little as 2 hours at room temperature following heat treatment.

2. Studies were carried out in the laboratory to determine the conditions under which the reactivation phenomenon occurred in skimmilk, whole milk, 20% fat and 38% fat cream.
3. By means of an H.T.S.T. tubular heater with a 16-second holding tube, portions of the various products were pasteurized at 165° to 240° F. In addition, a portion of each product was also long-time pasteurized at 145° F. for 30 minutes.
4. Immediately after pasteurization and when stored at 40° F., all the products showed negative phosphatase tests. When the samples were stored at 88° F., however, phosphatase reactivation was observed in H.T.S.T. treated products, but not in similar products pasteurized by the long-time method.
5. The minimum short-time temperature above which reactivation occurred in these products and the time of storage at 88° F. before the enzyme reactivated appeared to be a function of the fat content. Both 20% and 38% fat cream showed positive tests (reactivation) within 2 hours at 88° F. when heated for 16 seconds at 165° and at 180° F. and above, respectively. Skimmilk and whole milk, however, required longer storage at 88° F. after heat treatment (18 hours for the skimmilk, and 6 hours for the whole milk) and higher pasteurizing temperatures (210° and 195° F., respectively) before reactivation was noted.
6. Bacteriological studies made on all samples indicated that the reactivated phosphatase in the H.T.S.T. pasteurized products was not of bacterial origin.
7. The reactivation of phosphatase in dairy products after H.T.S.T. heating at temperatures used commercially indicates the possibility of making faulty interpretation of the phosphatase test when applied to H.T.S.T. products. By analyzing H.T.S.T. products immediately after heat treatment, or by insuring that the products have been properly refrigerated prior to testing, the danger of reactivated phosphatase interfering with the analysis is eliminated.

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COME-UP TIME METHOD OF MILK PASTEURIZATION. III. BACTERIOLOGICAL STUDIES[1,2]

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High pasteurization temperatures with very short or no intended hold are a definite trend in the dairy industry. Different investigators have compared their practicability with the times and temperatures originally specified by the U. S. Public Health Service. The authors have contributed timely information which was obtained by exposing some disease-producing bacteria to high temperatures for very short times. *Editor.*

Recently, there has been an increasing amount of interest in processes that can be used to pasteurize milk with shorter heating times than those used commercially today. With the use of higher temperatures it appears practical to depend entirely on the lethality of the come-up and cooling portions of the cycle to achieve adequate pasteurization. This would make it possible to completely eliminate the holding-time requirement normally contained in the definition of high-temperature short-time (HTST) pasteurization. Before standards can be set for new pasteurization processes, data are needed which would be valuable in suggesting these standards.

An instrument has been developed to heat milk rapidly. A description of this instrument, as well as results of a study on the effect of rapid heating on some milk properties, has been published *(5, 7)*. The object of this study was to evaluate the effect of heating times of less than one second on some bacteria that may be associated with milk.

REVIEW OF THE LITERATURE

Speck *et al. (10)* tested the heat resistance of *Micrococcus freundenreichii* in whole and chocolate milk. They found that there was no difference in heat resistance in the two products at 165°, 160°, and 155° F., whereas at lower temperatures longer times were required to destroy the test culture in chocolate milk than in whole milk. Exposure times as short as 19 seconds were examined. In a previous publication, Speck *(9)* found that a 1-second exposure of *M. freundenreichii* to 170° F. would give 99.99% destruction.

Shahani *et al. (8)* found that, in the range of 168° to 174° F., the same percentage of an inoculum of *M. freundenreichii* was destroyed as in laboratory

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pasteurization at 143° F. for 30 minutes. The heating time was 6.17 seconds and holding time was calculated to be 2.1 to 2.2 seconds. A Mallorizer heater *(4)* was used. Herreid *et al. (2)* modified this instrument so that the heating time was 4.0 seconds. The velocity of the milk through the heater and cooler was 23.78 feet per second. A holding tube of 1.0-in. inside diameter was used. Velocity in this tube was 1.49 feet per second. The heating and holding tubes were made of 0.25-in. stainless steel tubing. Tobias *et al. (11)* used the Mallorizer for studies on milk pasteurization at high temperatures. Heating times of 6.36 seconds, 2.36 seconds holding time, and 2.5 seconds cooling time (to 86.7° F.) were used for attaining a final temperature of 170.4° F. It was found that heating to 168.34° F., with a holding time of 2.36 seconds, was equivalent to 143° F. for 30 minutes as measured by the destruction of *M. freundenreichii*. A temperature of 169.7° F. was necessary to reduce the activity of phosphatase to 4 γ of phenol per milliliter.

Holland and Dahlberg *(3)* used rectangular tinned copper tanks, with dimensions of $20 \times 12 \times 1$ cm., to heat milk. The heat treatment was carried out in three stages, by means of water baths. In the first stage, temperature was held at 145°-150° F. to raise the temperature of the milk in the copper tank to 135°-140° F. The second bath was set at a temperature of 30° F. above that used for pasteurization temperature. The third bath was maintained at pasteurization temperature and was used as a holder. Cooling times were not defined. Heating times were determined for four final temperatures, from an initial temperature of 140° F. The heating times in seconds were 3.0, 5.5, 8.2, and 10.0 for final temperatures of 150°, 160°, 170°, and 175° F., respectively. It was found that, with a final heating temperature of 175° F. and no intended holding time, suspensions of 325,000 per milliliter of *Escherichia coli* were completely destroyed and cream volume was reduced 27% after 4 hours refrigeration. The phosphatase test showed 0.02 γ of phenol per 0.5 ml. of milk, and the rennet coagulating time was increased by 85%. Complete destruction of *E. coli* was achieved at a minimum temperature of 172.5° F. when no intended holding time was used.

EXPERIMENTAL PROCEDURE

Raw milk was obtained from the University of Massachusetts herd, pumped into the holding tank of the instrument, and forced through the small-bore (7/64 in. inside diameter) stainless steel tubing by air pressure. As the milk passed through the tube, it was heated to the desired temperature by heat exchange from the small-bore tube. The tube was heated by electrical resistance. Samples were collected by introduction of the milk into a precooled liquid medium. In all cases, the temperature of the mixture of precooled medium and heated milk was below 135° F. at the time of collection. A holding time of 0.05 second was inherent in the process. A detailed description of the instrument used in this study has been published *(5)*.

The bacteria studied in the investigation were *Escherichia coli*, ATCC ♯9637, *Streptococcus pyogenes* ATCC ♯7017, *Salmonella typhosa* ATCC ♯9498, *Shigella paradysenteriae* ATCC ♯9580, *Corynebacterium diphtheriae* ATCC ♯296, and

an unidentified species of micrococcus designated MS 102. The results with the MS 102 organism have been published previously *(7)*.

Escherichia coli ATCC #9637. Stock cultures were grown on Brain Heart Infusion Agar (BHI, Difco) and daily transfers of this organism were made to freshly prepared slants. Incubation was at 98.6° F. for 24 hours. A 24-hour slant culture was used to inoculate plates of BHI agar. The organisms, after 24 hours incubation at 98.6° F., were harvested by washing the growth from the plate with sterile distilled water. This suspension was filtered through Reeve Angel Filter paper #235 to remove large clumps. The optical density of the filtered suspension was adjusted to give the desired concentration of bacteria. Autoclaved milk was used in this study, with the exception of determinations which were made to check the validity of comparing results obtained in autoclaved milk with those of raw milk.

Samples were taken from the instrument by direct injection of the treated milk containing the test organisms into lactose broth. A series of 12 replicate samples was obtained for each temperature tested. Gas in any of the 12 replicate samples, after incubation at 98.6° F. for 48 hours, was considered as a positive test for *E. coli*. Positive tubes were spot-checked by streaking on Eosin Methylene Blue Agar (Difco). The procedure with raw milk was identical to that above with the exception that all samples were streaked on Eosin Methylene Blue Agar after 24 hours of incubation in lactose broth. Total counts before heat treatment were determined by standard plate-counting procedures in all cases.

The mean temperatures for sterilization (100% destruction) of suspensions of *E. coli* in autoclaved milk, with heating times of 0.25 second and 0.5 second above 135° F. were 174.5° and 172.3° F., respectively. This organism was also processed in raw milk to check the validity of applying data obtained in autoclaved milk to data from raw milk. Results from a series of ten trials, with a 0.5-second heating time, gave a mean temperature of 171.7° F. for 100% destruction of *E. coli*. An initial concentration of 8×10^6 organisms per milliliter was used throughout the study on this organism.

Streptococcus pyogenes ATCC #7017. *S. pyogenes* stock cultures were maintained by daily transfer on Brain Heart Infusion Agar (Difco). Stock suspensions of this organism were made with a technique similar to that for *E. coli*. The filtered suspension of organisms was suspended in raw milk. Azide Dextrose Broth was used as the recovery medium. The organisms were incubated in this medium for 24 hours and then transferred to Azide Dextrose Agar. Typical colonies in this meduim were considered as positive for *S. pyogenes*. Triplicate samples were used for each temperature investigated in this series.

With an initial concentration of 3×10^6 organisms per milliliter, and heating times of 0.25 second and 0.5 second above 135° F., the mean respective temperatures required for sterilization of *S. pyogenes* suspended in raw milk were 162.6° and 158.9° F. Since the thermal resistance of this organism appeared low, it was used to check the lethality of the preheating tube of the instrument to determine whether a preheat temperature of 135° F. had appreciable lethality. Preheat temperatures of 135° and 140° F. were tested. The heater section of

the tube was not heated and functioned only as a holding tube. The holding time was 0.5 second. With an initial count of 6×10^5 cells per milliliter, it was found that this process caused 8% reduction (from 6×10^5 to 5.5×10^5 organisms per milliliter) in count with a preheat temperature of 135° F., and 18% reduction (from 6×10^5 to 4.9×10^5 organisms per milliliter) when the final preheater temperature was set at 140° F. The vehicle used was raw milk.

Shigella paradysenteriae ATCC ♯9580. The inoculum was prepared in a manner similar to that for *E. coli* and suspended in raw milk. The organisms were recovered in Brain Heart Infusion Broth and incubated for 24 hours. Duplicate loop transfers were made to MacConkey's Agar (Difco) and Salmonella Shigella Agar (Difco). The appearance of typical white colonies on either medium was considered as a positive test for *S. paradysenteriae*. Colonies were spot-checked by agglutination with homologous antisera. Triplicate samples were processed for each temperature tested.

Concentrations of 5×10^6 organisms per milliliter required mean temperatures of 162.8° and 162.0° F. for 100% destruction of *S. paradysenteriae*, with respective heating times of 0.25 second and 0.5 second above 135° F., when raw milk was used as the suspension fluid.

Salmonella typhosa ATCC ♯9498. The inoculum was prepared as for *E. coli* and suspended in raw milk. Samples were taken from the instrument and inoculated into Selenite broth (Difco). After 24 hours incubation at 98.6° F., these samples were transferred to Bismuth Sulfite Agar and incubated for 48 hours. Typical colonies on this medium were considered as positive for *S. typhosa*. Colonies were spot-checked by agglutination procedures. Triplicate samples were processed for each temperature tested.

With *S. typhosa*, a heating time of 0.25 second produced 100% destruction, with a mean temperature of 165.1° F. A mean final temperature of 162.8° F. was required to sterilize suspensions of *S. typhosa* when a 0.5-second heating time was used. The initial concentration used for this organism was 3×10^6 cells per milliliter and the vehicle used was raw milk.

Corynebacterium diphtheriae ATCC ♯296. The inoculum was prepared as with *E. coli*. Samples were taken from the machine and inoculated into Brain Heart Infusion Broth. The primary recovery medium was incubated at 98.6° F. for 24 hours. Transfers then were made to Potassium Tellurite Agar plates. All samples were taken and processed in triplicate. The presence of typical colonies on potassium tellurite agar was considered as positive for *C. diphtheriae*. Colonies were checked by microscopic examination of the morphology of the individual cells.

Heating times of 0.25 second sterilized suspensions of *C. diphtheriae* at mean temperatures of 161.4° and 157.4° F., respectively. An initial concentration of 1.2×10^6 organisms per milliliter was used for this study. The vehicle used was autoclaved milk, since it was found that micrococci normally present in raw milk would multiply rapidly in the recovery medium. This would result in the suppression of *C. diphtheriae*. As a result, attempts to use raw milk as the suspending fluid failed to disclose the thermal resistance of *C. diphtheriae*.

TABLE 1

Temperatures required for complete destruction of five organisms with 0.25 second heating time Preheat temperature 135° F. Holding time 0.05 second

Organism	Vehicle	No. Trials	Mean	Range
			(° F.)	(° F.)
E. coli	Autoclaved milk	10	174.5	173.8-175.6
S. pyogenes	Raw milk	13	162.6	161.6-163.8
Sh. paradysenteriae	Raw milk	12	162.8	162.0-163.8
Sal. typhosa	Raw milk	21	165.1	164.1-166.1
C. diphtheriae	Autoclaved milk	16	161.4	158.3-163.7

TABLE 2

Temperatures required for complete destruction of test organisms with 0.5 second heating time Preheat temperature 135° F. Holding time 0.05 second

Organism	Vehicle	No. Trials	Mean	Range
			(° F.)	(° F.)
E. coli	Autoclaved milk	12	172.3	171.5-173.3
E. coli	Raw milk	10	171.7	171.0-172.4
S. pyogenes	Raw milk	12	158.9	158.4-160.0
Sh. paradysenteriae	Raw Milk	12	162.0	160.9-163.0
Sal. typhosa	Raw milk	12	162.8	162.0-163.8
C. diphtheriae	Autoclaved milk	10	157.4	155.7-158.5

The results reported above show that this organism has less heat resistance than that reported in a preliminary study *(6)*. The discrepancy apparently resulted from a pseudo-diphtheriae present in the raw milk. A summary of the results of the organisms studied is shown in Tables 1 and 2.

Statistical significance of results. The results of this investigation show that there is very little difference in the temperature required to kill a given organism or to inactivate phosphatase between the 0.25 and 0.5 second heating periods. Accordingly, a Standard Error Test for Significance was performed by the method of Velz *(12)* to determine whether the difference in temperatures required by the 0.25 and 0.5 second heating times was significant.

Values were computed for the 99% confidence level and compared with the difference in the arithmetic means of the 0.25 and 0.5 second heating times (Table 3). In all cases, the difference in the means was found to have significance at the 99% confidence level.

Equivalent times at 143° F. Heating-curve determinations were made for heating times of 0.25 second and 0.5 second, with final temperatures of 150°, 160°, 170°, 180°, 190°, and 200° F. It was found that the heating curve for the heating tube used in this instrument was essentially linear.

Equivalent times at 143° F. were calculated for heating times of 0.25 and 0.5 second with a 0.05 second holding time. This was done by drawing a thermal-death-time curve with an assumed z[3] value of 9, using an F_{143}[4] value, or process

[3] Slope of the thermal death time curve expressed in terms of the number of °F traversed by the curve in one log cycle.

[4] Destruction time at 143° F.

TABLE 3

Summary of statistical analysis of thermal destruction data for test organisms and phosphatase, with 0.25 and 0.5 second heating times

Organism or enzyme	Standard error of difference	99% confidence limit (3 SE)	Actual difference
Phosphatase	0.64	1.92	3.9
MS 102	0.26	0.78	1.6
E. coli	0.33	0.99	2.2
S. pyogenes	0.25	0.75	4.9
S. paradysentariae	0.24	0.72	0.8
S. typhosa	0.31	0.93	2.3
C. diphtheriae	0.54	1.62	4.0

value of 143° F. for 30 minutes. Lethal rates were computed for heating times of 0.25 and 0.5 second with a 0.05 second holding period, for final heating temperatures of 150°, 160°, 170°, 180°, and 200° F. From these, lethality curves were drawn and total lethality was determined by the graphical method *(1)*. Results are shown in Table 4. For example by calculation, it was found that, with a heating time of 0.25 second and a 0.05 second holding time, a final temperature of 190° F. with this process had 6.51 times the lethality of 143° F. for 30 minutes.

For comparative purposes, the lethality of the above heating procedures may be expressed in terms of holding time at 143° F., as is shown in Table 5.

TABLE 4

Total lethality ($z = 9$) of 0.5 and 0.25 second heating times, with 0.05 second holding time for final heating temperatures of 150°, 160°, 170°, 180°, 190°, and 200° F.

Final heating temperature	0.25 second heating time	0.5 second heating time
(° F.)	(units)[a]	(units)
150	0.00038	0.00059
160	0.0039	0.0055
170	0.0411	0.059
180	0.510	0.626
190	6.51	8.22
200	71.40	101.0

[a] One unit is equivalent in lethality to a process of 143° F. for 30 minutes.

TABLE 5

Sterilizing values (F_{143})[a] of heating times of 0.25 and 0.5 second with a 0.05 second holding time and final temperatures of 150°, 160°, 170°, 180°, 190°, and 200° F.

Final heating temperature	0.25 second heating time	0.5 second heating time
(° F.)	(min.)	(min.)
150	0.0114	0.0177
160	0.117	0.165
170	1.23	1.77
180	15.3	18.8
190	195.3	246.6
200	2142.0	3030.0

[a] Conventional holding pasteurization of 30 minutes at 143° F. taken to have $F_{143} = 30$ minutes.

For example, it was found that a final heating temperature of 190° F., with 0.25 second heating time and 0.05 second holding time, was equivalent in lethality to 143° F. for 195.3 minutes.

DISCUSSION

The investigation of the effect of short heating times on bacteria indicate that heating times, with no intended holding time, had sufficient lethality to completely kill the suspensions of bacteria which were inoculated into milk. A temperature of 175.6° F. was found sufficient to completely kill suspensions of *Sal. typhosa*, *Sh. paradysenteriae*, *S. pyogenes*, *C. diphtheriae*, and *E. coli*, when suspended in milk and heated for 0.25 second. All initial concentrations of the organisms tested were in excess of 1×10^6 per milliliter.

The heat resistant organisms (MS 102) were found to have a heat resistance considerably greater than that of the other organisms tested. Temperatures as high as 192.9° F., with a heating time of 0.25 second, were found necessary to produce 99.9% destruction of the organism *(7)*.

Phosphatase was previously found to be inactivated at a mean temperature of 182.4° F. with a heating time of 0.25 second *(7)*. This study indicates that phosphatase may still be used as a satisfactory indicator of the effectiveness of pasteurization, since the temperature found necessary to inactivate phosphatase was 6.8° F. higher than that required to kill any of the organisms tested, with the exception of the heat-resistant MS 102. This would make it possible to use phosphatase as an indicator of satisfactory pasteurization and still provide an adequate margin of safety.

The temperature found sufficient to kill a given organism with a 0.25 second heating time was found to be only slightly higher than that required to kill the same organism with a 0.5 second heating time. Accordingly, a significance test was performed, and it was found that all differences were significant at the 99% confidence level.

An attempt was made to completely eliminate the holding time in this process. However, because of the transition from final heating to cooling, complete elimination of the holding time could not be accomplished. The holding time for the bacterial studies was approximately 0.05 second and was in addition to the reported heating or come-up time. Cooling was accomplished by introducing the hot fluid into a precooled liquid. Since final temperatures of the liquid were always under 135° F., a calculation of the cooling time involves a consideration of the time required for any particle of fluid to reach thermal equilibrium with the surrounding fluid. It was believed that this time would be so short as to be negligible.

The heating curve for the heating tube used in this study was found to be essentially linear. That is, a given particle of milk was heated at a constant rate as it passed through the heating tube. This fact makes calculations of the amount of lethality in the heating cycle easily transformed mathematically to holding time.

When the lethality of this process is compared with that of 143° F. for 30 minutes, it is found that a final heating temperature of 181.5° F., with a 0.5-second heating time and a 0.05-second holding time, is comparable to 143° F. for 30 minutes in lethality, if an assumed z value of 9 is used. With 0.25 second heating time, and the same holding time and z value, it is found that 183° F. is comparable in lethality to 143° F. for 30 minutes. A review of the literature resulted in very little information on the z values of pathogens that may be found in milk. However, on the basis of existing data, a z value of 9 was selected as probably representative of the vegetative cells found in milk.

The results from the investigation of the effect of rapid heating on milk properties and bacteria indicate that pasteurization of milk can be accomplished without intended holding times. It is apparent that rapid heating rates have sufficient lethality for the production of a safe milk and in addition do not have an adverse effect on the product. In addition to the study on milk, the instrument has been used to sterilize suspensions of vegetative cells, as well as spores, when these suspensions were suspended in a gelatin diluent. It was found that sterilization of bacteria could be accomplished without appreciable alteration of pH, viscosity, surface tension, and specific gravity of the suspensions. Suspensions of viruses have been sterilized with this process, and the practicality of using this type of heating for bacterin production is under investigation at the present time.

Other applications wherein this type of processing might prove valuable include the sterilization of pharmaceutical products, elimination of hepatitis virus from the blood serum, processing of fluid food products, and basic information on the thermal resistance of vegetative cells and spores of bacteria in a variety of suspending fluids.

SUMMARY

Suspensions of *Escherichia coli* required mean temperatures of 174.5° and 172.3° F. for sterilization in autoclaved milk, with heating times of 0.25 and 0.5 second, respectively. Under the same conditions, a heating time of 0.5 second destroyed *E. coli* with a mean temperature of 171.7° F. when raw milk was used as the suspending fluid.

Mean temperatures of 162.6° and 158.9° F., for heating times of 0.25 and 0.5 second, were found sufficient to sterilize suspensions of *Streptococcus pyogenes* in raw milk.

Suspensions of *Shigella paradysenteriae* in raw milk were sterilized by mean temperatures of 162.8° and 162.0° F., with heating times of 0.25 and 0.5 second, respectively.

With heating times of 0.25 and 0.5 second, temperatures of 165.1° and 162.8° F., respectively, were found sufficient to sterilize suspensions of *Salmonella typhosa* when suspended in raw milk.

Suspensions of *Corynebacterium diphtheriae* were sterilized in heating times of 0.25 and 0.5 second, with respective mean temperatures of 161.4° and 157.4° F., when autoclaved milk was used as the suspending fluid.

Holding times of 0.05 second were inherent in the process and necessarily must be added to the reported heating or come-up time.

In conclusion, it appears from this investigation that a satisfactorily pasteurized milk can be produced by a process that uses fast heating and cooling rates without any intended holding time, by using pasteurization temperatures easily attainable with existing modern equipment.

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ENDOCRINE FACTORS INFLUENCING THE INTENSITY OF MILK SECRETION. A. ESTROGEN, THYROXINE, AND GROWTH HORMONE

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The total yield of a dairy cow during a lactation period is dependent on the total number of epithelial cells that are stimulated to growth during the first two-thirds of pregnancy and the initiation and maintenance of the intensity of milk synthesis in each of these cells. The role of three hormones—estrogen, thyroxine, and a growth hormone—in the regulation of the intensity of secretion during the normal declining segment of the lactation is described. *Editor.*

After parturition, the secretion of milk increases for a period of 1 to 2 months, reaches a peak or maximum rate of production, then declines gradually. The rate of decline in individual cows varies greatly. Some cows are said to be very persistent, whereas others decline rather rapidly. Gaines *(12)* defined persistency as the degree with which the rate of secretion is maintained as lactation advances. An exponential equation has been found to accurately describe the rate of decline of milk secretion of nonpregnant cows *(3)*. It was shown further that a superabundance of feed is stored as depot fat and does not appreciably affect the characteristic course of the declining curve *(17)*. On the other hand, underfeeding and several environmental, managerial and physiological factors, including pregnancy *(4)*, may decrease persistency.

Under uniform conditions, it is believed that the control of persistency of milk secretion is primarily dependent upon the rate of secretion of certain hormones that can influence the intensity of milk secretion in the individual cells of the udder. A second factor of importance would then be the gradual involution and loss of epithelial cells with the advance of lactation.

The total yield of milk of a dairy cow during a lactation period is dependent upon the total number of epithelial cells, which are stimulated to growth during the first two-thirds of pregnancy, and the initiation and maintenance of the intensity of milk synthesis in each of these cells.

In the preceding paper were described the endocrine factors which induced the growth of the mammary gland of sterile heifers and initiated the secretion of

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milk. It was shown that their maximum milk secretion closely approached that which might have been expected had these heifers calved normally *(19)*.

The object of the present paper is to report upon the effect of certain hormones that are believed to play a role in the regulation of the intensity of secretion during the normal declining segment of the lactation curve (18). The three hormones considered at this time are estrogen, thyroxine, and growth hormone.

Estrogen. The primary hormone stimulating the synthetic activity of the mammary gland cells is believed to be the lactogenic hormone of the anterior pituitary *(20)*. When this hormone is injected into animals with grown, but not lactating, mammary glands, the secretion of milk is quickly initiated. It is believed to be necessary throughout the entire period of lactation. When the rate of secretion of this hormone declines, the intensity of secretion of milk in the individual cell declines. It has been shown in many experimental animals that the amount of lactogenic hormone in the pituitary increases dramatically just before and after parturition and then gradually declines with the advance of lactation. The rise and decline of this hormone parallels the rise and decline of the milk secretion curve *(14)*.

It was shown further by work in this laboratory that the estrogenic hormone stimulated the secretion and content of the lactogenic hormone of the pituitary *(14)*. As a result of these observations the theory was advanced that the initiation and increase of lactation just before and after parturition was due to the rise in the secretion of the lactogenic hormone which, in turn, was stimulated by the rise in the secretion of the estrogenic hormone in late pregnancy.

Experimental proof of the theory of the role of estrogen in the stimulation of the lactation process was presented *(13, 19)*. It was shown that the injection of estrogen into heifers in which the glands were grown but not lactating induced the initiation and secretion of large quantities of milk. On the basis of these observations, it was suggested that if estrogen stimulated the functional activity of the acidophilic cells of the anterior lobe of the pituitary to secrete the lactogenic hormone during the rising segment of the lactation curve, then it might be possible to maintain an intense secretion of the lactogenic hormone after the period of maximum milk production by optimal administration of estrogenic hormone during the normal declining segment of the lactation curve and thus prevent the normal decline in milk secretion. Evidence that estrogen will prevent the decline in milk secretion will be presented.

Thyroxine. Extensive research has demonstrated that the intensity of milk secretion is controlled in part by the hormone of the thyroid gland, thyroxine *(2)*. If less than an optimal rate of thyroxine is being secreted in dairy cattle, the rate of milk production will be depressed below the potential possibilities of the cow. By increasing the amount of circulating thyroid hormone in such animals, either by injection of thyrotropic hormone *(5)* or by the injection or feeding of thyroxine, the intensity of milk secretion can be increased.

Thyroxine is a general body metabolic stimulant. It has been shown to favorably influence feed consumption; the rate and volume of blood flowing to the

udder; the composition of blood, especially the precursors of milk; cellular activity of the mammary gland cells; certain endocrine gland cells and body cells in general *(2)*.

Thus, dairy cattle which respond to the injection or feeding of the thyroid hormone by increased secretion of milk indicate, thereby, that their own secretion rate of this hormone is less than optimal in relation to the size of the udder (number of secreting cells) and to the secretion of other essential hormones.

The object of the present experiment was to determine in each of the heifers the extent to which the secretion of thyroxine was limiting the intensity of milk secretion. This was of particular interest since the actual thyroxine secretion rate of these heifers is being measured with radio-active iodine. The results of this latter study will be presented later.

Growth hormone. In recent years the marked effect of the growth hormone upon established lactation has been demonstrated *(1, 5-11, 15, 16, 21)*. It has been suggested that the primary effect of this hormone is due to the favorable effects it exerts upon the protein, carbohydrate, and fat precursors of milk. In the present study, interest in the effect of its action in individual cows was considered, that is, to what extent the intensity of milk secretion was limited by the endogenous secretion of the growth hormone.

Experimental method. The heifers induced to lactate experimentally were used in the present experiment *(19)*. When these heifers declined in milk production for a period of 2 weeks, the feeding of estrogen was begun. Diethylstilbestrol was premixed with finely ground cellulose (Cellu-flour), then mixed with the concentrate grain mixture so that each animal received 10 mg. per head per day in 5-mg. portions twice per day for a total period of 4 weeks. In a few cases the hormone was given again in late lactation.

Immediately after the treatment with diethylstilbestrol, the heifers were started on L-thyroxine at the rate of 0.4 mg. per 100 lb. live weight per head per day injected subcutaneously. The thyroxine was dissolved in sodium hydroxide, then neutralized and made up to the appropriate level with distilled water. The duration of the thyroxine treatment varied from 4 to 9 weeks. In addition to thyroxine, heifers No. 310 and 580 were injected with graded dosages of 5 to 20 units of thyrotropin plus radioiodine during the last 4 weeks of treatment.

The growth hormone was injected for a period of 7 days at the rate of 50 mg. per day. The immediate and continuing effect of the hormone was observed in individual cows in varying stages of lactation.

Effect of estrogen treatment. The addition of 10 mg. of diethylstilbestrol per day to the grain mixture of five heifers was started when the animals showed 2 weeks of declining production. The treatment was continued for 4 weeks (Table 1, Figures 1 and 2). It will be noted that in some animals the decline in milk yield continued for 1 week, followed by a tendency for the decline to be arrested and a slight increase to occur by the fourth week. Thus, in several of the heifers, the average milk production at the end of 4 weeks of treatment was equal to or higher than the production during the last week preceding the treat-

TABLE 1

Effect of feeding 10 mg. diethylstilbestrol daily on milk production

Herd No.	Breed	Average milk yield at maximum	Average milk yield at week preceding treatment	Average milk yield last week of treatment	Change in milk yield	Effect on decline
		(*lb/day*)	(*lb/day*)	(*lb/day*)	(%)	
310	Jersey	18.2	15.3	15.3	0.0	Arrested
580	Jersey	22.9	20.7	21.9	+ 5.8	Arrested
402	Holstein	33.3	30.1	26.9	− 11.8	Arrested
583	Jersey (3 glands)	12.2	11.8	12.1	+ 2.5	See Fig.
585	Jersey	18.2	17.1	17.6	+ 2.9	See Fig.
544	Jersey	29.6	16.3	18.2[a]	+ 11.7	Arrested

[a] Fed 20 mg. diethylstilbestrol per day.

ment. In Holstein No. 402, the decline the first week of treatment was not offset by a subsequent rise in production; there was, however, a tendency for the further decline in yield to be arrested. In cow No. 3596, thyroxine was administered first, followed by a period of a combination of thyroxine and diethylstilbestrol.

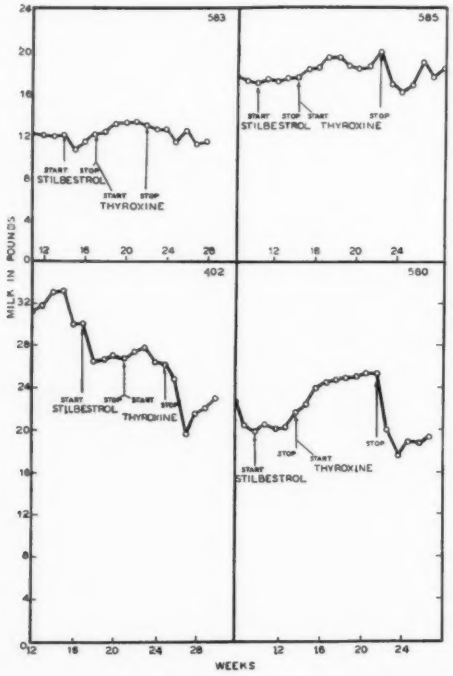

FIG. 1. The effect on the intensity of milk secretion of the feeding of diethylstilbestrol at the rate of 10 mg/ per day and of thyroxine injected at the rate of 0.4 mg/100 lb. body weight per day to 4 lactating dairy cows.

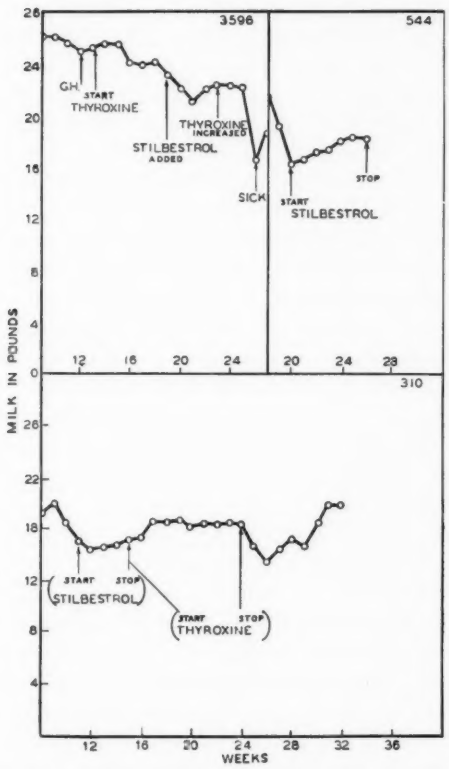

FIG. 2. The effect of diethylstilbestrol and thyroxine on milk production.

In this animal, the decline continued for 2 weeks, then increased and plateaued for a period of 4 weeks. A decline then occurred during a period of sickness. Cow No. 544 was fed 20 mg. per day of diethylstilbestrol some weeks after the peak of production (20th week of lactation). The decline was arrested, followed by a gradual increase in milk yield.

Later in the lactation period, several cows were again fed diethylstilbestrol to observe the effect on the decline in milk yield in late lactation. Cow No. 580 in the 40th week of lactation began a period of regular decline during a period of 5 weeks. After diethylstilbestrol feeding was started the decline was arrested; a slight increase followed, which was maintained for a period of 10 weeks of feeding.

Cow No. 585 in her 37th week of lactation had shown a decline in milk yield during a period of 6 weeks. She was started on diethylstilbestrol the 43rd week of lactation, when she was producing 10.5 lb. of milk per day. She then gradually increased to a maximum of 12.9 lb., which was sustained for a period of 9 weeks without decline.

Although these data are limited both as to the number of cows available and to the duration of treatment, they suggest that low levels of estrogen fed at the beginning of the declining phase of lactation will tend to arrest the rate of decline of milk secretion. In two cows, it was shown that even in late lactation the feeding of diethylstilbestrol arrested the decline in milk production and caused maintenance of the level for periods of 9 to 10 weeks. Further studies through extended periods of the lactation cycle are required to demonstrate the continuing value of low levels of the estrogens on the intensity of milk secretion.

If further research should confirm these observations on the arrest of the decline in milk secretion through the mediation of low levels of fed estrogen, the question of the passage of estrogen into the milk secreted will be raised. This problem is now being investigated and will be reported in detail later. Comparisons of the milk of nonpregnant cows with cows fed 10 mg. daily of diethylstilbestrol indicate that estrogen fed at this level does not pass into the milk in assayable quantities.

The second question will be the effect of feeding diethylstilbestrol at this level to pregnant cows. Preliminary reports on the feeding of 10 mg. of diethylstilbestrol to beef heifers indicate that it does not interfere either with subsequent conception or with the maintenance of pregnancy.

Effect of thyroxine treatment. At the conclusion of the 4 weeks of diethylstilbestrol feeding, the estrogen treatment was discontinued and daily injections of L-thyroxine were initiated in five heifers. This treatment was continued for 4 weeks in two heifers (No. 402 and 583) and for 8 or 9 weeks in three heifers (No. 310, 580, and 585). In addition to thyroxine, No. 310 and 580 were injected with graded dosages of 5, 10, 15,, and 20 units of thyrotropin (Armour & Co.), plus iodine I^{131} during the last 4 weeks of treatment in order to study the effect of thyrotropin on the uptake of I^{131} by the thyroid. This phase of the study will be reported later. As would be expected, the milk yields were not significantly affected by the addition of thyrotropin in the presence of thyroxine. Two additional cows (No. 544 and 3596) were tested.

The daily injection of thyroxine at the rate of 0.4 mg. per 100 lb. body weight produced variable increases in average weekly milk production during the injection period (Table 2 and Figures 1 and 2). Cow No. 310 showed an immediate rise in production, which was then maintained for a period of 8 weeks. After the cessation of injections, there was a decline for 2 weeks, followed by a rise above the previous level of milk production, and up to the peak level.

Cow No. 580 showed a rise during the last week of diethylstilbestrol and was continued on thyroxine during an 8-week period. It is interesting to note that her production at the peak of her initial rising segment (experimentally induced) was 22.9 lb. of milk, whereas after 8 weeks on thyroxine she reached a peak of 25.4 lb. per day. It is believed that this indicates that her productivity was seriously limited by her normal thyroxine secretion rate. When supplied an increased amount of exogenous thyroxine, she demonstrated increased ability to secrete milk. Upon the withdrawal of thyroxine, she showed a precipitous

TABLE 2
Effect of injecting L-thyroxine daily on milk production

Herd No.	Breed	Milk yield at maximum	Milk yield week preceding treatment	Milk yield last week of treatment	Change in milk yield	Duration of treatment
		(*lb/day*)	(*lb/day*)	(*lb/day*)	(%)	(*weeks*)
310	Jersey	18.2	15.3	16.5	+ 7.8	8
580	Jersey	22.9	21.9	25.4	+16.0	8
583	Jersey (3 glands)	12.2	12.1	13.4	+10.7	4
585	Jersey	18.2	17.6	20.6	+17.0	9
402	Holstein	33.3	27.2	26.4	− 3.0	4
544	Jersey	29.6	25.4	26.7	+ 5.12	4
3596	Guernsey	26.3	25.1	24.2	− 3.70	6
			24.2	22.1[a]	− 9.5	4
			22.1	18.7[b]	−15.4	6

[a] Thyroxine was continued with 10 mg/day of diethylstilbestrol added.
[b] Thyroxine increased to 0.45 mg/100 lb. body weight with diethylstilbestrol.

decline in milk production for 2 weeks, followed by a slight rise as her own thyroid glands resumed activity.

Cow No. 583, a low producing cow with only three functional quarters, showed a moderate increase in milk production during a 4-week period on thyroxine. She also increased in milk production above the initial maximum experimentally induced. Upon withdrawal of the thyroxine, she alone showed no immediate decline in milk production.

Cow No. 585 showed a rise in milk production upon thyroxine administration exceeding the initial maximum experimentally induced. Her greatest production was reached the ninth week of injection. Upon withdrawal, lactation declined for 2 weeks, and then increased.

Cow No. 544 was started on thyroxine at the time she leveled off at the peak of production. She then showed a further rise during a period of 3 weeks. After a temporary change in milkers, she declined temporarily, thus preventing determination of her full response.

Cow No. 3596 was placed on thyroxine alone at the rate of 0.35 mg. per 100 lb. weight for a period of 6 weeks. She showed very little response during the first 3 weeks, then declined. At the beginning of the seventh week, 10 mg. daily of diethylstilbestrol was added to the feed. Production declined slightly for 3 weeks, then increased slightly for 2 weeks. At this time, the thyroxine level was increased to 0.40 mg. per 100 lb. body weight, stilbestrol being continued. The increase had no effect upon milk production. The cow became sick temporarily and her milk production declined. This cow showed no response to thyroxine at either level.

The response of the group of cows to thyroxine was variable. Cow No. 580 showed the greatest response, increasing in production above that attained at the peak of experimental production. Cows No. 585 and 583 also increased in lactation above the previous peak although the yield was not as great. Cow No. 310 showed only a slight increase, but the yield was well maintained. Cow No. 3596 was unresponsive. These data are interpreted as indicating that the response

in milk production to thyroxine may be used to determine the degree to which endogenous thyroxine may be a limiting factor in the productive capacity of individual cows.

When thyroxine was suddenly withdrawn, there followed a decline in milk production for about 2 weeks until the cow's thyroid gland resumed normal function. It is interesting to note that the recovery in milk yield after thyroxine withdrawal was greatest in cows with the smaller responses.

Effect of growth hormone. It will be noted that the injection of growth hormone was continued for a single week in contrast to the continued administration with the other hormones. The cow most responsive to growth hormone was No. 580 (Table 3). Milk yield increased during the week of treatment from

TABLE 3

Effect of the injection of growth hormone upon milk production

Herd No.	Breed	Milk yield at maximum	Milk yield week preceding treatment	Milk yield at maximum	Change in milk yield	Duration of stimulation[a]
		(*lb/day*)	(*lb/day*)	(*lb/day*)	(%)	(*weeks*)
310	Jersey	18.2	14.3	16.8	+17.5	3
580	Jersey	22.9	19.5	25.7	+31.7	8
583	Jersey (3 glands)	12.2	11.8	15.5	+31.4	3
402	Holstein	33.3	20.7	26.5	+28.0	2
585	Jersey	18.2	12.7	12.8	+ 0.7	No stimulation
160	Jersey	27.3	18.7	22.5	+20.3	3
544	Jersey	29.6	20.6	20.5	− 0.5	No stimulation
3596	Guernsey	26.3	25.7	25.1	− 2.4	No stimulation

[a] Time in weeks before return to production preceding treatment.

19.5 lb. to 25.7 lb. per day and continued at this level for a period of 8 weeks. This production was equal to her response to thyroxine and exceeded her initial maximum. Starting in the 57th week of lactation she was again injected with growth hormone. She increased about 2 lb. per day, which gain was sustained for a period of 4 weeks.

Cow No. 583 increased from 11.8 lb. per day to a peak of 15.5 lb. after the injection of growth hormone starting the 40th week of lactation. The increase was sustained for 3 weeks followed by a decline to the starting level. This production was the highest level reached by this cow, exceeding that stimulated by thyroxine.

Cow No. 402 in her 36th week of lactation was given growth hormone. During the preceding week she averaged 20.7 lb. per day. She increased to a maximum of 26.5 lb. in 2 weeks. Unfortunately, milkers were changed at this time, which resulted in a drop in milk production.

Cow No. 310 in her 44th week of lactation was producing 14.3 lb. of milk per day. Upon the injection of growth hormone, production increased to a peak of 16.8 lb. within 3 weeks, then declined.

Cow No. 585 was injected with growth hormone on the 53rd week of lactation when she was producing 12.7 lb. of milk. She showed no response during the week of injection or during the following week.

Cow No. 160 was in the 16th week of lactation, producing 18.7 lb. of milk per day, when injected with growth hormone. She increased her production gradually during a period of 3 weeks to a maximum of 22.5 lb., then returned to the preinjection level.

Cow No. 544 showed no increase in milk yield when growth hormone was administered during the 16th week of lactation. Cow No. 3596 was injected with growth hormone during the 12th week of lactation without a stimulating effect being noted.

Of the eight cows injected with growth hormone at various stages of lactation, five showed a response, which was sustained for 2 to 8 weeks even though the hormone was administered for a period of a week. Three cows showed no response. The variation in the response is interpreted as indicating the degree to which the intensity of milk secretion is limited by the rate of endogenous secretion of the growth hormone.

Discussion. The inheritance of the capacity for lactational milk production in the cow is dependent upon the inheritance of the rates of secretion of the various endocrine glands that supply the hormones necessary for the growth of the udder and the maintenance of the intensity of milk secretion. The present report is concerned with a study of the latter problem in a group of heifers whose udders were stimulated to growth by the injection of progesterone and estrogen, then stimulated to the initiation of lactation by estrogen *(19)*.

A number of hormones have been shown to influence the intensity of milk secretion in cattle. If any one or all of these hormones are being secreted in less than optimal amounts, the administration of the hormone in question should stimulate an increase in the milk yield of the cow up to the point where some other hormone secretion rate would become the limiting factor in productivity.

By injecting or feeding a succession of hormones during the declining segment of the lactation curve, it should be possible to determine which of the endocrine glands are limiting the productivity of individual cows. It is suggested that such a technique should make it possible to determine the genetic causes of the variability in milk yield of individual cows and of the progeny of dairy sires. Thus, in the selection of sires and dams, the indices of productivity would not be total production, but the adequacy of the rate of secretion of the hormones which make possible high production.

Thus, cows that fail to respond to the additional hormone would be considered to be secreting adequate amounts of the hormone. In other words, their productivity was not limited by the endogenous rate of secretion of the hormone. Cows that do respond to the administration of the hormone show, thereby, that their production was being limited and if the inherited rate of secretion of the hormone had been greater, their rate of milk secretion normally would have reached the level which the added hormone made possible.

Analysis of the results of the study. From a consideration of the results of the previous *(19)* and present studies, the following conclusions are reached in regard to each cow (Table 4).

TABLE 4

Effect of various hormones on intensity of milk secretion

Herd No.	Breed	Milk yield at maximum	Response to hormone treatment					
			Diethylstilbestrol		Thyroxine		Growth hormone	
			Maximum milk yield	Change in yield	Maximum milk yield	Change in yield	Maximum milk yield	Change in yield
		(*lb./day*)	(*lb.*)	(%)	(*lb.*)	(%)	(*lb.*)	(%)
310	Jersey	18.2	15.3	+ 0.0	16.5	+ 7.8	16.8	+17.5
580	Jersey	22.9	21.9	+ 5.8	25.4	+16.0	25.7	+31.7
402	Holstein	33.3	26.9	−11.8	27.2	− 3.0	26.5	+28.0
583	Jersey (3 glands)	12.2	12.1	+ 2.5	13.4	+10.7	15.5	+31.4
585	Jersey	18.2	17.6	+ 2.9	20.6	+17.0	12.8	+ 0.7
544	Jersey	29.6	18.2	+11.7	26.7	+ 5.1	20.5	− 0.5
160	Jersey	27.3					22.5	+20.3
3596	Guernsey	26.3			24.2	− 3.7	25.1	− 2.4

Cow No. 310, a Jersey heifer, was induced experimentally to secrete a maximum of 18.2 lb. of milk daily. This was estimated to equal 65.9% of the maximum production of her close relatives. The feeding of diethylstilbestrol arrested the decline in milk production. This is interpreted as indicating that the lactogenic hormone was beginning to decline in secretion rate. The response to exogenous thyroxine was slight but persistent, indicating that the thyroxine secretion rate was only a slight limiting factor in milk production. The response to growth hormone was greater in advanced lactation, indicating that the secretion of this hormone was a more severe factor limiting milk production.

Cow No. 580, a Jersey heifer, secreted a maximum of 22.9 lb. of milk per day, which was estimated to be 137.1% of the maximum production of her close relatives. In spite of this evidence of production, she responded well to diethylstilbestrol, thyroxine, and growth hormone, indicating that her secretion rates of these hormones were limiting her productivity. With the latter two hormones, she exceeded markedly the initial maximum production. It is clear that this cow's productivity was seriously limited by a lack of endogenous secretion of thyroxine and growth hormones.

Cow No. 583, a Jersey heifer, had only three functional glands, yet produced at her maximum 73.1% of the maximum production of her close relatives. Her response to exogenous hormones was similar to that of No. 580 except that she did not respond quite as well to thyroxine although she exceeded her initial maximum production in both cases. It is believed that aside from a lack of gland tissue, her two endocrine gland deficiencies were thyroxine and growth hormone secretions. Heifers No. 580 and 583 were by the same sire.

Cow No. 402, a Holstein heifer, produced at her maximum 93.0% as much milk as her dizygotic twin, which calved in the University herd. Her twin had two records and showed a marked lack of persistency and in her second lactation dropped off so fast that she was discarded. No. 402 showed this same tendency, but diethylstilbestrol arrested the decline after the first week. Her response to thyroxine was short-lived, but her response to the growth hormone was marked

at the 36th week of lactation. She was diagnosed as being deficient in the secretion of lactogenic and growth hormones.

Cow No. 585, a Jersey heifer, produced 127.3% of the maximum production of her close relatives. Her rate of decline of lactation was small (high persistency). Her response to diethylstilbestrol in early lactation was slight. In late lactation, when her decline was marked, she showed a better response, which was maintained for 9 weeks. She showed a good response to thyroxine, exceeding her initial maximum production. She showed no response to growth hormone. Her endocrine deficiencies were diagnosed as chiefly thyroid with lactogenic hormone playing an increasing role in later lactation.

Cow No. 544, a Jersey heifer, was brought into lactation experimentally and reached a maximum production of 29.6 lb. of milk per day during the 7th week, which was estimated to be about her production had she calved normally. She showed a slight response to thyroxine, no response to the growth hormone, but a good response to diethylstilbestrol fed at the 20 mg. level. She appeared to indicate a lactogenic hormone deficiency with advanced lactation.

Cow No. 160, a Jersey heifer, reached a maximum weekly production of 27.3 lb. per day. She was tested only with the growth hormone and showed a good response (20.3%), indicating that her productivity was limited by her growth hormone secretion rate.

Cow No. 3596, a Guernsey heifer, reached a maximum weekly production of 26.3 lb. Her response to thyroxine and growth hormone was negative, indicating that other hormones limited her productivity.

SUMMARY

1. A group of dairy heifers whose udders were grown and subsequently induced to secrete milk experimentally were allowed to reach their maximum production without further treatment. When they began to decline in milk yield, each heifer was treated with three hormones, one after the other, to determine their effect upon the intensity of milk secretion.

2. It was assumed that if the hormone administered either arrested the normal decline or increased the intensity of milk secretion it would indicate that the animal's endogenous secretion of the hormone was limiting its milk secretion capacity.

3. It was shown that the daily feeding of 10 mg. of diethylstilbestrol for a period of 4 weeks tended to arrest the decline in milk yield and in some animals caused a slight increase in milk yield during the period. In two cows in late lactation the prolonged feeding of this hormone caused the maintenance of the level for periods of 9 to 10 weeks.

4. It was suggested that these data indicate that low levels of estrogen tend to prevent the decline in the secretion of the lactogenic hormone of the pituitary.

5. The injection of L-thyroxine at the level of 0.4 mg. per 100 lb. body weight for periods of 4 to 9 weeks in some heifers produced a marked rise in milk yield, whereas in others the effect was less or nil.

6. These data are believed to indicate the degree to which the endogenous rate of secretion of thyroxine was limiting the milk producing capacity of the individual heifer.

7. When the injection of thyroxine was stopped suddenly, the yield of milk declined rapidly for about 2 weeks then increased to the level which might have been expected at that stage of lactation. During periods of exogenous thyroxine administration, the secretion of enogenous thyroxine is prevented but returns rapidly to the normal rate when thyroxine injections are stopped.

8. The injection daily of 50 mg. of growth hormone for a period of 1 week stimulated a marked increase in milk yield in five of eight cows injected, which increase was sustained for periods of 2 to 8 weeks. Three cows showed no response.

9. The variation in the response to growth hormone was interpreted as indicating the degree to which the intensity of milk secretion was limited by the rate of endogenous secretion of the growth hormone.

10. On the basis of the responsiveness of each animal to the three hormones administered, an analysis of the genetic-endocrine causes of their variability in milk production was suggested. Data similar to this in regard to the dams and to the progeny of dairy sires would make possible matings of animals, not on the basis of total production, but on the adequacy of the rate of secretion of the hormones that make possible high milk production.

ACKNOWLEDGMENT

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EFFECT OF AUREOMYCIN ON YOUNG DAIRY CALVES RAISED IN A NEW ENVIRONMENT [1]

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Aureomycin increased the weight and feed utilization efficiency of Holstein and Jersey calves and more so when they were kept in a sanitary environment. *Editor.*

Several studies *(3, 4, 9)* have shown that certain antibiotics were ineffective in stimulating the growth of germ-free chicks. Recently however, Luckey *(10)* reported that germ-free birds receiving oxytetracycline, 25 mg. per kilogram of diet, grew more rapidly (3 to 7%) than unsupplemented birds.

Many workers *(2, 5, 8, 11, 12, 13)* have observed also that chicks or pigs that were raised in new uncontaminated quarters failed to show any antibiotic growth response. Healthy chicks or pigs in new quarters grew as well or faster than those fed antibiotics in old quarters. The disease level of these animals was thought to be the factor responsible for the lack of growth stimulation to antibiotic administration.

On the other hand, Hill *et al. (6)* observed that a small growth response was noted in birds in new quarters after 3 weeks, which might be attributed to a gradual contamination of the new quarters. More recently, Hill and Larson *(7)* obtained greater daily gains and higher feed utilization efficiency in baby pigs held in isolation units from birth to 24 days of age when receiving supplementation of 10 p.p.m. aureomycin.

Bartley and coworkers *(1)* reported that control calves in a new barn grew as well as aureomycin-fed calves in an old barn. However, aureomycin-fed calves at the new barn grew at a significantly faster rate (approximately 10% at 16 weeks of age) than the control animals.

Since a newly built calf barn with new pens, hayracks, and feeding utensils was available, a comparison was made to determine the effect of aureomycin on calves raised in a new uncontaminated environment with those raised in an old environment.

EXPERIMENTAL PROCEDURE

Sixty-one newborn purebred male dairy calves (30 Holsteins and 31 Jerseys) were used in this study. Twenty-nine calves (18 Holsteins and 11 Jerseys) were

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housed in the old barn and 32 calves (12 Holsteins and 20 Jerseys) in a newly constructed barn furnished with new equipment and feeding utensils. Calves at each barn were divided at random into three groups according to breed. Group I was fed a basal ration (Table 1) and served as the control; Group II

TABLE 1
Experimental rations used

	Groups	
	I and III	II
	(*lb.*)	(*lb.*)
Cotton seed meal	35.0	35.0
Ground yellow corn	31.3	31.3
Rolled oats	15.0	15.0
Wheat bran	10.0	10.0
Soybean oil meal	5.5	5.5
Bone meal	2.0	2.0
Salt	1.0	1.0
Quadrex X[a]	0.2	0.2
Aurofac 2A[b]	—	0.5

Chemical analysis of the rations (A.O.A.C. method)

	Groups	
	I and III	II
	(%)	(%)
Crude protein	24.20	23.80
Ether extract	3.50	3.80
Crude fiber	7.80	7.20
Water	10.40	9.70
Ash	6.90	7.10
N.F.E. (by difference)	47.20	48.40

[a] Supplied by the Nopco Chemical Co., Harrison, N. J. Contains vitamin A, 10,000 U.S.P. units and vitamin D_2, 1,250 U.S.P. units per gram.

[b] Supplied by the American Cyanamid Co., Pearl River, N. Y. Contains 3.6 g. aureomycin per pound.

received aureomycin orally (50 mg. daily in the milk, and a 0.5% level of Aurofac 2A[2] in the calf starter), and Group III was administered weekly 400 mg. aureomycin in sesame oil by intramuscular injection.

The animals were kept in individual pens on a cement floor covered with fresh shavings. All calves were fed colostrum for the first 3 days of life. From the fourth day up to 28 days of age they were fed whole milk daily from a nipple pail at a rate of 1 lb. per 10 lb. body weight in two feedings, half in the morning and half in the evening. Calf starter rations (Table 1) were fed ad libitum from the fourth day of age until a maximum of 5 lb. was being consumed daily by the Jerseys and 6 lb. by the Holsteins. Good quality alfalfa hay was fed ad libitum, beginning at 7 days of age, and clean water was available at all times.

Feed consumption was recorded daily and all calves were weighed at weekly intervals to 12 weeks of age.

[2] Supplied by the American Cyanamid Co., Pearl River, N. Y.; contains 3.6 g. aureomycin per pound.

RESULTS AND DISCUSSIONS

Growth and feed efficiency. The body weight gains and feed utilization are summarized in Table 2. All antibiotic-treated calves in both barns made greater

TABLE 2

Effect of aureomycin administration on the growth and feed utilization efficiency of dairy calves

Treatment and age	Av. daily gains per calf (*lb.*)				TDN/lb. gain			
	Barn				Barn			
	Old		New		Old		New	
	Jerseys	Holsteins	Jerseys	Holsteins	Jerseys	Holsteins	Jerseys	Holsteins
0–4th week								
control	0.30	0.49	0.43	0.58	0.71	0.55	0.49	0.26
oral	0.60**	0.77**	0.60**	0.76**	0.22	0.28	0.30	0.18
injected	0.38**	0.67**	0.55**	0.96**	0.58	0.32	0.28	0.23
0–8th week								
control	0.53	0.87	0.69	0.78	2.11	1.64	1.60	1.47
Oral	0.91**	1.29**	0.91**	1.05**	1.10	1.37	1.33	1.21
injected	0.78**	1.07**	0.88**	1.13**	1.50	1.41	1.39	1.11
0–12th week								
control	0.77	1.15	0.83	1.13	2.47	2.18	2.30	2.94
oral	1.21**	1.53**	1.16**	1.42**	1.64	1.98	1.75	1.73
injected	1.00**	1.31**	1.10**	1.43**	2.14	2.05	1.88	1.66

** Highly significant at 1/or at $P < 0.01\%$ level of probability.

body weight gains and consumed their feed more efficiently than did their respective control animals at 4, 8, and 12 weeks of age. These differences were statistically significant at $P < 0.01$. The results obtained in this study indicate that regardless of the type of environment in which calves are raised, aureomycin administration increases growth rate. Bartley et al. *(1)* also reported a growth stimulation in calves raised in a new environment when fed aureomycin. These workers obtained an increased gain of 349% of the average first week's weight for calves receiving 45 mg. of aureomycin daily, 314% for those receiving 15 mg. aureomycin, and 291% for the control calves. Similar results were reported by Hill and Larson *(7)* with swine. They observed that aureomycin exerted a growth-promoting effect in pigs delivered by hysterectomy and raised in an isolated area free from recognized swine disease.

Lillie *et al. (8)* found that chicks in old quarters showed the effect of antibiotic feeding at 1 week of age, but in new quarters there was no effect until 2 to 4 weeks. In the present calf study, an increase in growth rate was obtained as early as the second week from aureomycin feeding in a new barn. At the end of the second week of age, the antibiotic-fed animals had gained 0.54 lb. daily in the new barn as compared to 0.26 lb. in the old barn. There were small differences in body weight gains of the antibiotic-treated calves in the two barns at the end of 4 weeks of age.

A few reports *(1, 8)* have indicated that animals in new quarters fed no antibiotics grew as rapidly as animals in the old quarters fed antibiotics. This

study did not confirm such findings. The aureomycin-fed calves in the old barn grew faster than the untreated calves raised in the new barn. These body weight gains were approximately 20, 37, and 33% in favor of the aureomycin-fed calves at the end of 4, 8, and 12 weeks of age, respectively.

In general, the feed efficiency of calves raised in the new barn was higher in comparison to similar groups at the old quarters, irrespective of treatments (Table 2). The Holstein calves showed a better feed efficiency than the Jersey calves. In both barns all aureomycin-fed calves had a higher feed efficiency than their respective control animals. The difference among groups at the new barn was more pronounced than among those at the old barn.

Scours. The occurrence of scours was greater in the old barn than in the new barn when all calves were considered (Table 3). At the old barn the intramuscu-

TABLE 3

Effect of aureomycin upon incidence of scours in a new environment (scours-days per calf)

		Jerseys			Holsteins		
		Week			Week		
Treatment	Barn	0–4	5–8	9–12	0–4	5–8	9–12
Control	Old	(3)[a] 2.00	(1) 1.00	0.0	(1) 1.00	(2) 5.5	0.0
	New	(5) 4.40	0.00	0.0	(3) 5.34	(1) 3.00	0.0
Oral aureomycin 50 mg./day	Old	(3) 3.00	(1) 4.00	(1) 19.00	(2) 1.50	(1) 6.0	0.0
	New	0.0	0.0	0.0	0.0	0.0	0.0
Injected aureomycin 400 mg./wk.	Old	(2) 2.00	0.0	0.0	(3) 2.00	0.0	(1) 2.00
	New	(1) 6.0	0.0	0.0	(1) 3.00	0.0	0.0

[a] Figures in parentheses indicate number of animals scouring.

lar injection of aureomycin seemed to prevent scouring but the oral administration of the antibiotic was not as effective. The situation in the new barn was different. In both antibiotic groups (groups II and III) scouring was very slight and only the calves that were intramuscularly injected with aureomycin scoured.

Leucocyte counts. White blood cell counts in the antibiotic-treated calves were reduced as compared to the control animals at the old barn. In both breeds, especially the injected-treated calves, lower white blood cell counts were observed as the calves grew older and were most pronounced at 12 weeks of age. However, the difference in white blood cell counts at this age was not statistically significant between treatments.

White blood cell counts of the calves at the new barn revealed no differences among groups at all stages of collection, 4-week interval (Table 4). This is in

TABLE 4

Effect of aureomycin administration on white blood cell count of dairy calves ($\times 10^3$/cu. mm.)

Treatment	Control		Oral		Injected	
Barn	Old	New	Old	New	Old	New
Jerseys, No.	3	8	4	6	4	6
0–4th week	9.53	8.89	7.81	8.09	6.94	9.13
5–8th week	9.88	8.83	8.15	7.98	5.85	9.83
9–12th week	9.75	9.04	5.70	8.13	5.00	9.79
Holsteins, No.	6	4	6	4	6	4
0–4th week	10.48	8.86	10.63	10.45	8.93	10.04
5–8th week	9.97	8.95	8.13	10.10	5.92	9.31
9–12th week[a]	7.05	9.08	6.68	9.65	4.75	9.35
Combined						
0–4th week	10.16	8.88	9.69	9.04	8.27	9.50
5–8th week	9.94	8.86	8.14	8.83	5.90	9.63
9–12th week	8.06	9.08	6.31	8.74	4.84	9.62

[a] Old Barn — 5 animals per group.

agreement with the report of Voelker *et al. (14)*, who observed no significant difference in the total leucocyte and differential leucocyte counts in Holstein calves fed aureomycin and other antibiotics as compared to control calves. If leucocyte counts are used as criteria for an infection-free condition, the data collected in this paper indicate that all calves were in the same degree of health at all times. From this assumption, the data obtained imply that aureomycin exerts a growth-promoting effect in calves even in the absence of a disease level. Further work is needed to justify this observation since no accurate criterion for an absolute infection-free condition is available at the present time.

SUMMARY

The effect of oral-fed and intramuscular-injected aureomycin on young calves raised in a new uncontaminated and in an old environment was investigated. The data show that regardless of what type of environment calves are raised in, aureomycin administration statistically ($P < 0.01$) increased body weight gains and feed utilization efficiency at 12 weeks of age. An earlier stimulation in growth was observed in calves raised in a new barn than in those raised in an old barn. The growth response from antibiotic administration was detected earlier in the oral-fed groups than in the injected-treated animals. In the new barn, the growth-promoting effect of aureomycin administration was detected as early as 2 weeks, whereas in the old barn the effect was not observed for 4 weeks. This finding indicates that the cleanliness and sanitary conditions of the environment favor a greater and more effective growth-stimulation response of aureomycin feeding.

There was no significant difference in white blood cell counts among the antibiotic and control groups in the old and new quarters, indicating that all calves apparently were in a similar state of health.

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GENETIC AND ENVIRONMENTAL FACTORS IN THE DEVELOPMENT OF THE AMERICAN RED DANISH CATTLE[1, 2]

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In 1939, Red Danish bulls were introduced into herd service in Michigan, where they were rotated from herd to herd. A study of the resulting grading-up project has been made. Among the factors considered were butterfat production, lethal defects, calf mortality, and sterility of the cows. *Editor.*

Young Red Danish bulls were introduced from the USDA herd at Beltsville, Maryland, into Michigan during 1939 and several years immediately following. County bull associations were formed, and the bulls were used extensively in farm herds of various dairy breeds. The bulls were rotated from herd to herd. The program, by reason of repeated back-crosses to the Red Danish bulls, became a grading up project. Favorable results and growing interest among the dairymen enrolled led to the establishment of the American Red Danish Cattle Association in 1948. An open herd book was set up, and provision was made to accept third-cross females and fourth-cross males for registration. Records of production on female ancestors were made a requirement for registration. An investigation of the results of the program was begun by Cranek in 1950 and continued by Thompson in 1953.

PROCEDURE

Data were obtained through the cooperation of breeders, agricultural extension workers, and the American Red Danish Cattle Association. In the first part of the investigation (by Cranek), milk and butterfat lactation records of 270 to 365 days duration were adjusted to 305-day values by factors developed by Dickerson *(3)* and to maturity by standard multiplicative age correction factors *(10)*. In the second part of the investigation (by Thompson), additive correction factors for age at calving, previous calving interval, length of lactation period,

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[2] The data are from theses submitted by L. J. Cranek, Sr., and N. R. Thompson in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Michigan State University, 1952 and 1955, respectively.

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and year of calving first were developed from the unadjusted and uncorrected raw data by a least squares procedure *(7, 9)*. These correction factors then were used to adjust all apparently normal lactation records of 200 to 365 days length to a mean age of 64 months, a mean previous calving interval of 12.7 months, a mean length of lactation period of 292 days, and to the approximately median year 1946. In all cases, the lifetime average of each cow was used as a measure of her producing ability.

The general method for developing additive correction factors from results of the least squares analysis (Table 1) was to *(a)* find the mean for a given

TABLE 1

Least squares estimates of the effects of environmental factors on butterfat production[a]

Factor	Component	Effect
		(lb.)
Year of calving[b]	Linear	6.5**
Month of calving[c]	Linear	−0.770
	Quadratic	0.009
Age at calving[d]	Linear	10.012**
	Quadratic	−0.067*
Previous calving interval[d]	Linear	1.955**
Length of lactation[e]	Linear	0.994**

* Significant at 5% probability level.
** Significant at 1% probability level.
[a] Based on an intracow analysis of 2,301 records.
[b] Years 1941 to 1952, inclusive.
[c] Months denoted by ordinal numbers, 1 for Jan., 2 for Feb., etc.
[d] In months.
[e] In days.

variable, *(b)* calculate the expected deviation in butterfat yield for each unit deviation of the variable, and *(c)* reverse the sign of the deviation for butterfat. As an example, the mean previous calving interval was 12.7 months and the regression of butterfat yield on previous calving interval was 1.955 lb. per month. Thus the deviation for a 12-month interval was minus 1 lb. (to the nearest whole pound) and the correction factor, plus 1 lb.

The choice of an appropriate least squares analysis presented some problems. Ideally, it would have been desirable to fit constants for all variables. Practically, this was not feasible, since the number of equations needed would have been more than 2,000 and thus well beyond the capacity of most or all computational systems. Therefore it was necessary to reduce the number of equations and terms to a point where a solution was feasible. Henderson *(9)* showed how to set aside the constants for cows. The relation of age at calving to production may be computed as a curvilinear regression. Inspection of one-way tabulations of butterfat yield and month of calving suggested that a parabolic curve, with low points in June-July and high points in December-January, might give a reasonable first approximation of the trend. Similarly, it was assumed that linear regressions would give reasonable estimates of the relations between butterfat yield on the one hand and number of days milked and length of preceding calving

interval on the other. Thus it was deemed necessary to fit constants only for years, and even this proved to be not absolutely necessary. In fact, only the linear component (Table 1) among the deviations for years was used in adjusting the data in the second part of the investigation.

The mathematical model assumed was

$$Y_{ijkmnp} = \mu + a_i + b_j + d_1(M_{ik} - \overline{M}) + d_2(M^2_{ik} - \overline{M^2}) + d_3(A_{im} - \overline{A}) + d_4(A^2_{im} - \overline{A^2}) + d_5(K_{in} - \overline{K}) + d_6(L_p - \overline{L}) + e_{ijkmnp},$$

in which Y_{ijkmnp} is the record of the i^{th} cow calving in the k^{th} month of the j^{th} year, at the m^{th} age (in months), and with the n^{th} previous calving interval (in months) and the p^{th} length of lactation period (in days). The a_i's stand for deviations (from the population mean) of real producing abilities of individual cows, the b_j's for deviations associated with year of calving, and the d's symbolize partial regression of butterfat yield on the respective variables; μ is the population mean, and e_{ijkmnp} is the residual or error deviation from this mean.

It will be noted that the mathematical model includes no deviation for herd effect. Inasmuch as cows typically stay in one herd for their entire productive lifetimes, it was assumed that the intracow analysis which resulted from setting aside the constants for cows was also approximately intraherd.

The equations for the least squares analysis were solved by procedures such as have been reported by Henderson *(9)* and by Harvey *(7)*.

Heritability of butterfat production (Table 2) was estimated by the method of intrasire regression of daughter on dam *(13)*, by using 413 daughter-dam pairs from the first-cross and foundation generations. Repeatability of butterfat

TABLE 2

Estimation of heritability of butterfat yield [a]

Item	Sums of squares for daughters	Sums of squares for dams	Cross products
Over-all uncorrected S.S. & S.C.P.	62,171,123	58,314,428	58,911,145
Correction factors	60,730,749	56,263,076	58,333,750
Within-sire corrected S.S. & S.C.P.	1,440,374	2,051,352	577,395

$$\overline{H} = 2\left(\frac{577,395}{2,051,352}\right) = 0.5629$$

Applying the formula by Laben and Herman *(11)*:

$$H = \overline{H}\left(r + \frac{1-r}{\overline{d}}\right) = 0.5629 \left(0.4274 + \frac{1-0.4274}{2.1036}\right)$$

$$= 0.3938 \pm 0.1129$$

[a] Based on 413 daughter-dam pairs with daughters by 31 sires.

H = single-record heritability.

$\overline{H}$ = Heritability based on average records.

r = repeatability.

$\overline{d}$ = average number of records for the dams.

TABLE 3

Estimation of repeatability of butterfat yield

Source of variation	Degrees of freedom	Mean square	Expectation of mean square
Total	743		
Between cows	236	1,026	$\sigma^2 + 3.72\,\sigma^2_c$
Within cows	507	272	σ^2

$\sigma^2_c = (1{,}026 - 272)/3.72 = 203$

$$\text{Repeatability} = \frac{203}{203 + 272} = 0.4274$$

production (Table 3) was estimated by the method of intraclass (in this case intracow) correlation *(18)* from 744 lactation records on 237 cows in the first-cross generation. Both heritability and repeatability of butterfat production were estimated from records that had been adjusted for age at calving, year of calving, previous calving interval (where this existed or was known), and length of lactation period. The formula by Laben and Herman *(11)* was used to reduce the heritability value to a single-record basis.

Butterfat production of the foundation groups and of the generations which resulted from repeated crossing to the Red Danish sires was compared both on a daughter-dam basis and over entire generation groups. Repeatabilities of length of calving interval and length of lactation period were estimated by the method of intraclass correlation (*loc. cit.*).

The repeated use of a relatively few Red Danish sires led to inbreeding in the second-cross and later generations. The IBM procedure by Hazel and Lush *(8)* was followed in calculation of the inbreeding coefficients.

RESULTS AND DISCUSSION

Effects of environmental factors on butterfat production. The effect of month of calving on butterfat production (Table 1), as estimated by the fitting of a curvilinear regression, was not significant. The failure to be significant may have been due to the particular coding scheme used (January, 1; February, 2; . . . . . ; December, 12). The outcome does not necessarily imply that no effect whatever was exerted but only that the reduction in variance from fitting this particular regression was not significant. The literature, in general, suggests that cows calving in the fall and early winter months under temperate zone conditions yield more milk than those calving at other times of the year *(14)*.

The effect of previous calving interval on butterfat production (Table 1) was highly significant. The estimated gain of approximately 2 lb. butterfat for each additional month of calving interval is much less, however, than that reported by other workers *(5, 16)*. The effect of length of lactation period on butterfat production was estimated as 1.0 lb. more of butterfat for each additional day a cow was milked (Table 1). The effect of both previous calving interval and

length of lactation period, although estimated as linear regressions, may have departed significantly from linear relationships at the extremes. For example, the regression of butterfat yield on number of days milked would be definitely curvilinear at the peak of lactation.

The least squares analysis revealed a highly significant upward environmental trend of 6.5 lb. butterfat per cow per year (Table 1). This value (equal to 162 lb. FCM) is much greater than the 53 lb. FCM reported by Dillon *et al. (4)*. Bias due to selection and incomplete repeatability could have influenced this estimate of yearly environmental trend upward, providing the selection from lactation to lactation also was upward. The extent of such selection in the foundation groups is not known. In the first-cross and second-cross generations some selection did occur from lactation to lactation (Table 8), but the direction of this selection was not upward in every instance. The existence of a yearly environmental trend in the population investigated was not surprising. One of the requirements of the program was that production records be kept, and it was not unreasonable to expect increases in production as a result of improvements in feeding and management after the herds were placed on D.H.I.A. test.

Heritability and repeatability of butterfat production. Heritability of butterfat yield was estimated as 0.39 ± 0.11 (Table 2). This value is somewhat higher than a number of estimates from the literature. This may have resulted *(a)* from adjusting the individual lactation records not only for age and number of days milked but also for year of calving and previous calving interval and *(b)* from the fact that the Red Danish sires were rotated from herd to herd with the consequence that the intrasire regression of daughter on dam was not strictly intraherd. The result from adjusting for the two additional environmental effects (year of calving and previous calving interval) should have been to decrease the size of the denominator in the heritability fraction and thus increase its numerical value.

Repeatability of butterfat yield was estimated as 0.43 (Table 3). Inasmuch as the between-cow component included herd differences, the value 0.43 is a population estimate rather than intraherd.

Repeatability of environmental factors. The repeatability of length of lactation period was estimated as 0.17. The repeatability of calving interval was estimated as 0.086. The latter is somewhat less than the value for calving interval of 0.133 found by Legates *(12)* and about half as great as the values of 0.184 and 0.175 obtained by Rennie *(16)*.

Correlations among environmental factors. The simple intra-cow correlations among age at calving, previous calving interval, and length of lactation period are shown in Table 4. A slight tendency of calving interval to increase with age was shown by the small but significant positive correlation of 0.068 between age at calving and calving interval. Other intracow correlations among these factors were numerically small and not statistically significant.

Effects of the grading up program on butterfat production. The average adjusted butterfat production of each generation group is shown in Tables 5

TABLE 4

Within-cow correlations[a] among age at calving, previous calving interval, and length of lactation period

	Previous calving interval	Length of lactation
Age at calving	0.07*	0.06
Previous calving interval		−0.03

* Significant at 5% probability level.
[a] Based on 2,301 lactation records on 1,118 cows.

and 6. The records used in these comparisons were adjusted for effects of age at calving, year of calving, length of previous calving interval, and length of lactation period, as previously indicated in the discussion of procedure. It was assumed, in making comparisons among generations, that representatives of each generation had equal opportunity to be in each herd, and therefore that the possible bias from inclusion of herd differences in generation differences was minimized. Data from records on all cows (Table 7) showed a very highly significant increase ($P < 0.001$) from the foundation groups as a whole to the first-cross generation. The same was true for the Guernsey, Shorthorn, Brown Swiss, and mixed breed foundation groups, but not for the Jerseys. The gain from the Holstein foundation group to the first cross was significant ($P < 0.05$). No gain was apparent from first-cross to second-cross generations, but the increase from second to third cross was very highly significant ($P < 0.001$).

TABLE 5

Average butterfat production in foundation breed groups

Breed group	No. of cows	No. of records per cow	Average butterfat
			(*lb.*)
Guernsey foundation:			
All cows	236	2.22	339
Cows with daughters in first cross	107	2.68	359
Holstein foundation:			
All cows	163	2.01	375
Cows with daughters in first cross	67	2.58	384
Shorthorn foundation:			
All cows	178	2.02	333
Cows with daughters in first cross	65	2.35	346
Jersey foundation:			
All cows	67	2.52	382
Cows with daughters in first cross	31	3.29	382
Brown Swiss foundation:			
All cows	32	2.50	365
Cows with daughters in first cross	11	2.82	376
Mixed breed foundation:			
All cows	125	1.79	349
Cows with daughters in first cross	27	2.07	383
All foundation cows	801	2.10	351

TABLE 6

Average butterfat production in successive crosses to Red Danish sires

Generation group	No. of cows	No. of records per cow	Average butterfat
			(*lb.*)
First cross:			
All cows	703	2.22	379
Cows with Guernsey dams	139	2.17	389
Cows with Holstein dams	100	2.11	380
Cows with Shorthorn dams	83	2.19	367
Cows with Jersey dams	50	2.60	401
Cows with Brown Swiss dams	13	2.46	358
Cows with mixed breed dams	32	2.22	390
Cows with daughters in second cross	212	3.19	378
Second cross:			
All cows	411	1.99	377
Cows with dams in first cross	308	2.03	378
Third cross:			
All cows	123	1.60	389

On a daughter-dam basis, very highly significant increases ($P < 0.001$) occurred from Guernsey, Shorthorn, and Jersey foundation groups to first cross, but no apparent change was evident from Holstein or mixed breed foundation groups to their daughters. The first-cross daughters of Brown Swiss foundation dams showed a significant decrease ($P < 0.02$). No change at all was evident from first- to second-cross generations.

In general, the increases appeared to be large from foundation generations to first cross, virtually zero to second cross, and moderately small to third cross. The latter increase (from second to third cross) may be poorly estimated, since only 123 third-cross females were considered.

TABLE 7

Comparison of average butterfat production among generation groups

Generations compared	Av. gain or loss	Standard error of difference
	(*lb.*)	(*lb.*)
Gross basis (over all cows):		
All foundation vs. 1st cross	28	1.03 ($P < 0.001$)
Guernsey vs. 1st cross	40	1.48 ($P < 0.001$)
Holstein vs. 1st cross	4	1.77 ($P < 0.05$)
Shorthorn vs. 1st cross	46	1.70 ($P < 0.001$)
Jersey vs. 1st cross	− 3	2.39 ($P < 0.3$)
Brown Swiss vs. 1st cross	14	3.38 ($P < 0.001$)
Mixed breed vs. 1st cross	30	2.07 ($P < 0.001$)
First cross vs. 2nd cross	− 2	1.26 ($P < 0.2$)
Second cross vs. 3rd cross	12	2.29 ($P < 0.001$)
Daughter-dam basis:		
Guernsey vs. 1st cross	30	2.43 ($P < 0.001$)
Holstein vs. 1st cross	− 4	3.01 ($P < 0.2$)
Shorthorn vs. 1st cross	21	3.22 ($P < 0.001$)
Jersey vs. 1st cross	19	3.95 ($P < 0.001$)
Brown Swiss vs. 1st. cross	−18	7.45 ($P < 0.02$)
Mixed breed vs. 1st cross	7	5.22 ($P < 0.2$)
First cross vs. 2nd cross	0	—

The large increases from foundation generations to first cross are comparable to those secured by Fohrman *et al. (6)* and may be due in part to heterosis. Although the first-cross generation was approximately intermediate in butterfat production between the foundation groups and females in the Beltsville Red Danish herd, the failure of the second- and third-cross generations to continue the ascent in production at a comparable rate suggests that the first-cross group was higher in production than could be explained by intermediacy alone.

The increases from foundation generation groups to first cross appear to be related to the level of production of the foundation dams. Note, for example, that a large increase occurred in the progeny of the relatively low-producing Guernsey foundation group, but virtually no increase in the Holstein group (Tables 5, 6, 7).

The above comparisons between successive generations were made by the use of lifetime averages on individual cows. A question arises concerning the effects of selection on the group averages. The extent of selection from lactation to lactation in the foundation groups is not known. If effective selection had been achieved prior to the beginning of the grading-up program, it is possible that succeeding phenotypic performances of the remaining foundation cows gave biased estimates of their additively genetic values. All first-cross individuals (as well as those of subsequent generations) were supposed to have been production tested in D.H.I.A. for at least one lactation; this was stipulated in the program that included use of the Red Danish sires. Thus no selection whatever should have done prior to completion of first lactations, and the selection in subsequent lactations (Table 8) should not have biased the group averages of these crossbred generations either upward or downward. This would follow because

TABLE 8

Selection from lactation to lactation

Lactation	Per cent culled[a]	Selection differential[b]
		(*lb.*)
First-cross generation:		
First	39	4
Second	22	0
Third	12	1
Fourth	10	5
Fifth	8	1
Sixth	3	0
Seventh	4	10
Eighth	1	0
Second-cross generation:		
First	37	10
Second	32	3
Third	17	10
Fourth	9	− 8
Fifth	3	− 9

[a] Percentages based on original number completing first lactation.

[b] Difference between mean butterfat yield of cows retained for following lactation and mean of all cows completing current lactation.

the group averages are not simple averages of all individual lactation records but instead are averages of the individual lifetime averages of single cows.

Effects of inbreeding on milk and butterfat production. A total of 317 inbred females were observed. All were second- and third-cross individuals, and they represented progeny of 35 sires. Milk and butterfat records of the 317 cows were adjusted to 305-day mature equivalents by use of the standard factors mentioned earlier *(3, 10)*. The intrasire regression of production on inbreeding was minus 23.1 lb. milk and minus 0.3 lb. butterfat for each 1% of inbreeding. These regression values are somewhat lower than those reported by Nelson and Lush *(15)* but more nearly agree with the results of Bartlett and Margolin *(1)*. There was some evidence that the effect of inbreeding on butterfat production was not entirely consistent from sire to sire although herd differences may have added to the inconsistency. It should be noted that the base for inbreeding was a population of several breeds and their crossbred progeny, rather than a single breed.

Lethal defects revealed by inbreeding. Sixty-five calves in 27 herds were born with paralyzed hind quarters *(2)*. Forty-two calves in 11 herds were dead at birth and showed ankylosis and mummification. Both defects have been reported in the Red Danish cattle in Denmark, and the paralyzed condition has been traced to certain bulls of the breed *(17)*.

Pedigree studies of the sires used in the grading up program revealed 21 bulls to be heterozygous for the paralysis and 18 bulls heterozygous for the ankylosed condition. Chi-square tests of the observed and expected frequencies of normal and affected individuals suggested that each defect was due to a single autosomal recessive gene. It was estimated that 25% of the American Red Danish cattle were heterozygous for the paralyzed condition and 11% for the ankylosed condition.

Calf mortality. Records available on the foundation groups showed that only 0.7 to 2.3% of the calves were dead at birth. In contrast, the values for the first-, second-, and third-cross generations were 3.7, 7.7, and 9.3%, respectively. A part of the apparent increase could have been due to greater completeness of records in the crosses as compared to the foundation generations. Another part of the increase could have been due to the ankylosis defect. However, the increase in calf mortality began in the first-cross generation before inbreeding could have produced any homozygotes for this defect. Furthermore, the evidence of calf mortality did not increase with inbreeding.

Sterility in cows. Three categories of sterility were considered, namely *(a)* no conception from four or more services, *(b)* complete lack of heat periods, and *(c)* apparent conception followed by signs of heat three or more months later. In the foundation groups, only 13.1% were reported as being removed from the herd because of sterility of the above categories. However, 20.2% of the first-cross generation were removed for this reason, 25.8% of the second-cross generation, and 28.2% of the third. The incidence of sterility did not appear to be related to inbreeding.

SUMMARY

Genetic and environmental factors in the development of the American Red Danish cattle were investigated. The intrasire regression of production on inbreeding was -23.1 lb. milk and -0.3 lb. butterfat for each additional 1% of inbreeding. Two lethal defects, paralyzed hind quarters and ankylosis, appeared in the second cross to Red Danish sires. Calf mortality and cow sterility apparently increased with successive crosses.

Heritability of butterfat production was estimated as 0.39 ± 0.11, repeatability as 0.43. Average adjusted butterfat production in the foundation, first-cross, second-cross, and third-cross generations was 351, 379, 377, and 389 lb., respectively. Butterfat yield did not vary significantly with month of calving but increased 2.0 lb. ($P < 0.01$) for each additional month of previous calving interval and increased 1.0 lb. ($P < 0.01$) for each additional day of lactation period. An upward environmental trend of 6.5 lb. butterfat per cow per year ($P < 0.01$) also was found.

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STUDY OF SOME CHROMOGENS IN COW URINE [1]

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Neutral ether extracts of the urine of both pregnant and nonpregnant lactating cows were examined spectrophotometrically. Certain similarities between the chromogens of cow urine and the 17-ketosteroids present in human urine were noted. The urinary excretion pattern of these chromogens displayed a distinct diurnal rhythm. *Editor.*

The original object of this study was to measure photometrically the level of 17-ketosteroids in cow urine. In contrast to considerable work in this area in respect to humans *(13)*, there has been relatively little work done in respect to dairy cattle.

Marker *(11, 12)* reported on the isolation of the following steroids from pregnant cow urine: pregnanediol-3(a)-20(a), allo-pregnanediol-3(a)-20(a), allo-pregnanediol-3(b)-20(a), alpha and beta equistanol, androsterone, and dehydroisoandrosterone, only the latter two being 17-ketosteroids.

Meschaks *(14)* investigated photometrically the so-called "neutral steroid fraction" of the urine of cows, using both the Zimmerman and Pincus reactions, which were designed to measure the 17-ketosteroids in human urine. Although definite color development was obtained with these reactions, the absorption curves did not correspond to those obtained with androsterone.

Arrhenius *(2)* reported on the use of zinc and HCl for the hydrolysis of cow urine prior to extraction for 17-ketosteroids but presented no data which were critical in establishing the steroid nature of the extracts. Nor did Rosenkrantz *(20, 21)*, who reported on techniques for the extraction, separation, and purification of both 17-ketosteroids and estrogens from both equine and bovine urines, present data which would prove the steroid nature of the fractions.

Gassner *et al. (5)* isolated a fraction from the adrenal venous blood of cows which were administered ACTH intravenously. This fraction was androgenic by bioassay and gave the Zimmerman reaction for 17-ketosteroids.

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Mixner *(15)* and Mixner and Saunders *(16, 17)* in preliminary work reported on the extraction, separation, and spectrophotometric analysis of the so-called "neutral steroid" fractions of cow urine. Definite absorption spectra were obtained with the Zimmerman, Pincus, and Allen color reactions, but these spectra did not correspond to those obtained with the 17-ketosteroids.

Holcombe *(7)* has reported on the assay of neutral steroids and reducing corticoids in cow urine. He studied cows in various reproductive states and found close correspondence between the relative quantities of the two types of "steroids" present, but did not show the degree of specificity of the assay procedures.

Holtz *(10)*, working with extracts of cow urine similar to those used by Meschaks *(14)*, Mixner *(15)*, and Holcombe *(7)*, noted the presence of several ionone derivatives, which he identified as follows: (2,3,6-trimethyl-benzol)-acetone, 5-hydroxy-cis-tetrahydro-ionone, and 5-oxo-cis-tetrahydro-ionol; the identity of other ionone derivatives was not definitely established. Some of these compounds when introduced into the Zimmerman reaction gave absorption curves similar to those reported by Meschak *(14)* and Mixner *(15)*. With the Pincus reagent the 5-hydroxy-cis-tetrahydro-ionone gave a blue violet color with an absorption maximum at 550 mμ, an absorption spectra previously reported by Meschaks *(14)* and Mixner *(15)* for urine extracts. Holtz also demonstrated with some certainty the following three 17-ketosteroids: dehydroisoandrosterone, etiocholanolone, and 11-hydroxyandrosterone. In comparison with ionone derivatives, they were present in very small quantities. Holtz suggested that the ionone compounds are carotenoid derivatives from animal feed that is rich in carotene.

Holm and Firch *(8)* reported on the determination of 17-ketosteroids and 17-hydroxycorticosteroids from cattle urine by the use of the mild acid and glucuronidase hydrolysis procedures of Glenn and Nelson *(6)*.

This paper presents certain data relative to the hydrolysis, extraction, chromatography, and color reactions of these urine chromogens of cow urine.

METHODS AND MATERIALS

Color reactions. Three color reactions were used in this study, the Zimmerman reaction as modified by Holtorf and Koch *(9)*, the Pincus reaction *(18)*, and the Allen reaction *(1)*. These reactions have been used for the assay of urinary 17-ketosteroids in the human. A Beckman Model B spectrophotometer was used for all photometric determinations.

Extraction of urine. The chromogens, present in urine in the conjugated state, must be hydrolyzed before they can be extracted with lipid solvents. Acid hydrolysis and extraction methods similar to those described by Pincus *(19)* were used to prepare "neutral extracts." In some instances B-glucuronidase of bacterial origin was used for hydrolyzing the urine before extraction. The Arrhenius *(2)* hydrolysis procedure (zinc plus hydrochloric acid) also was tried.

Chromatographic separation. The method of Dingemanse *et al. (4)*, as used for separating 17-ketosteroids by means of an alumina column and a benzene-ethyl alcohol elution system, was modified for separating these chromogens.

RESULTS

Color reactions. Many neutral extracts of the urine of both pregnant and nonpregnant lactating cows were examined spectrophotometrically. The Zimmerman reaction absorption spectrum is characterized by high absorption of light in the near ultraviolet region (330 mμ) with low absorption in the region of 700 mμ (Figure 1). The 17-ketosteroids with this reaction have an absorption maximum at 510 mμ.

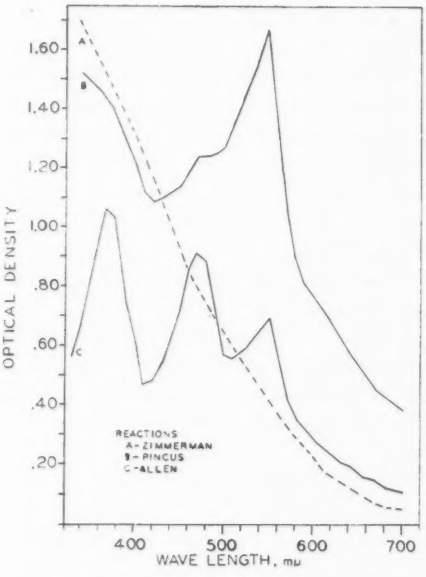

FIG. 1. Absorption spectra obtained when the Zimmerman, Pincus, and Allen color reactions are applied to neutral ether extracts of cow urine.

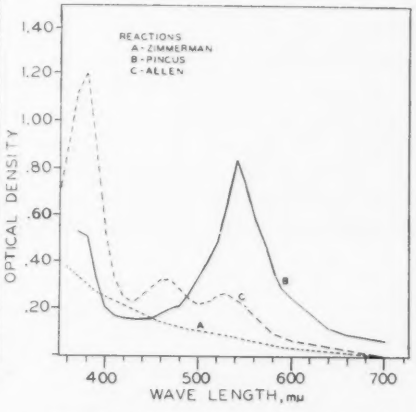

FIG. 2. Absorption spectra obtained with 5-hydroxy-cis-tetrahydro-ionone (Prelog's hydroxyketone G) using 5, 1, and 1 mg. respectively in the Zimmerman, Pincus, and Allen reactions.

When used with cow urine extracts the Pincus reaction has an absorption spectrum characterized by an absorption maximum at 540-550 mμ and another in the near ultraviolet region (Figure 1), in contrast to an absorption maximum at 610 mμ given by androsterone.

The Allen reaction has been used as a diagnostic aid for adrenal cortical tumors in man where dehydroisoandrosterone is found in the urine in abnormally high quantities. With this 17-ketosteroid an absorption maximum is found at 600 mμ. The neutral extracts of cow urine gave absorption maxima at approximately 370, 470, and 550 mμ (Figure 1). Androsterone gives absorption maxima with the Allen reaction at 390, 470, and 540 mμ *(1)*.

A pure sample of 5-hydroxy-cis-tetrahydro-ionone (Prelog's hydroxyketone G),[4] one of the two main fractions which Holtz *(10)* identified and separated from cow urine, was subjected to the three color reactions and gave absorption spectra (Figure 2) nearly identical to those already presented for extracts of cow urine (Figure 1).

To determine to what extent the three color reactions were measuring the same chromogen "complex" in cow urines, extracts were prepared from urine samples from 22 pregnant and nonpregnant lactating cows. These urines were acid-hydrolyzed and a neutral ether extract was prepared. All reactions were run in duplicate. Five ml. of urine equivalent was used in the Zimmerman reaction (510 mμ), 10 ml. in the Pincus reaction (550 mμ), and 2 ml. in the Allen reaction (470 mμ). Both reagent and urine blanks were used for color correction of the assay tubes. The following coefficients of correlation were calculated by the method of least squares *(22)* among the various estimates of chromogen concentration in which the optical density value was the unit datum: Zimmerman × Pincus, + 0.55; Zimmerman × Allen, + 0.78; and Pincus × Allen + 0.77, all highly significant statistically.

Hydrolysis of urine. Acid hydrolytic procedures that had been applied to the 17-ketosteroids in human urine were found to be suitable for releasing the urinary chromogens. In a trial experiment the quantity of concentrated HCl per 100 ml. of urine was varied from 7½ to 30 ml. and the time of boiling during hydrolysis from 7½ to 30 minutes. When the results were compared, by using the three color reactions (Table 1), the optimal conditions for hydrolysis were

TABLE 1

Study of variables associated with HCl hydrolysis of urine

	Optical density units								
	Zimmerman, 510 mμ at			Pincus, 540 mμ at			Allen, 470 mμ at		
HCl (volume %)	7-½ min.	15 min.	30 min.	7-½ min.	15 min.	30 min.	7-½ min.	15 min.	30 min.
7-½	0.71	0.81	0.82	1.99	2.02	1.63	0.68	0.97	0.92
15	0.85	0.87	0.86	1.93	1.80	1.24	1.14	1.04	0.85
30	0.89	0.78	0.69	1.40	0.83	0.64	0.82	0.50	0.48

found to employ 15 ml. of concentrated HCl per 100 ml. of urine in conjunction with a 10-minute boiling period. The use of zinc in conjunction with hydrochloric acid *(2)* as a hydrolyzing agent also was tried. Although much clearer extracts were obtained by this method, the recovery of chromogen was much less.

The use of B-glucuronidase of bacterial origin (optimal pH 6.5) as a hydrolyzing agent also was investigated. After a study of some of the variables (enzyme unitage, length of hydrolysis at 37° C.) influencing the recovery of chromogens, the following procedure was found to be very satisfactory.

[4] Obtained from Professor V. Prelog of the Laboratorium fur Organiche Chemie, Eidgenossische Technische Hochschule, Zurich, Switzerland.

1. To 100 ml. of urine, add 200 ml. of 0.3 *M* KH_2PO_4, 100 ml. of 0.3 *M* $Na_2HPO_4 . 11H_2O$, and 25,000 units of bacterial B-glucuronidase[5] (final pH 6.5).

[5] Sigma Chemical Company, Saint Louis.

2. Incubate for 2 days at 37° C., then adjust pH to 3.0 with approximately 30 ml. of 25% HCl.
3. Extract immediately with 400 ml. of ether; centrifuge to reduce emulsion if necessary.
4. Wash ether with 1 X – 100 ml. H_2O, 2 X – 100 ml. 15% Na_2CO_3, 3 X – 100 ml. 10% NaOH, and 2 X 100 ml. H_2O.
5. Control extraction for method blank is performed in the same way except for the substitution of water for the urine in the original step. Some chromogen development is exhibited by extracts of B-glucuronidase alone when assayed by the three color reactions.

These enzymatic studies were carried out in a manner similar to those reported by Cohen *(3)* for human urinary corticosteroids, 17-ketosteroids, estrogens, and pregnanediol.

Extracts of cow urine prepared by enzyme hydrolysis gave absorption spectra almost identical to those prepared by acid hydrolysis when the three color reactions were applied.

Ketonic, nonketonic separation of urine extracts. Ketonic and nonketonic separations were performed on neutral ether extracts of pregnant cow urines by using Girard's T reagent according to the method of Pincus *(19)*. The greater part of the chromogen was recovered in the nonketonic fraction as measured by the three color reactions (Table 2).

TABLE 2

Distribution of chromogens in ketonic, nonketonic separation of neutral ether extract of pregnant cow urine

Reaction	Wave length	Total chromogen in original extract	Total chromogen recovered in ketonic and nonketonic fractions	Distribution of recovered chromogen	
				Ketonic fraction	Nonketonic fraction
	(mμ)	(%)	(%)	(%)	(%)
Zimmerman	510	100	60.6	28.4	71.6
Pincus	550	100	66.7	35.7	64.3
Allen	470	100	73.8	23.1	76.9

Chromatographic separation of chromogens. The method of Dingemanse *(4)*, which utilizes flowing column chromatography with alumina as the adsorbent and benzene and ethanol as eluting agents, was modified for separating the chromogen "complex" into some of its constituents. The procedure as finally developed consisted of placing 12.5 g. of alumina in a chromatographic tube (18 mm. I.D.). Urine extract equivalent to 500 ml. of urine was taken up in 50 ml. of benzene.

The column was wet with benzene and the urine extract was added. This becomes fraction number 1. Elution of the column was as follows:

Fraction No.	Eluants
1	extract in 50 ml. benzene
2 – 5	4 X – 50 ml. benzene
6 – 15	10 X – 50 ml. of 0.1% ethanol in benzene
16 – 25	10 X – 50 ml. of 0.5% ethanol in benzene
26 – 31	6 X – 50 ml. of 1.0% ethanol in benzene
32 – 34	3 X – 50 ml. of 100% ethanol
35 – 36	2 X – 50 ml. 50% acetic acid in ethanol

The chromogen distribution among the various eluates was nearly identical as measured by the three color reactions, and accordingly only a plot of the Allen reaction data is presented (Figure 3). From this plot, evidence for the presence

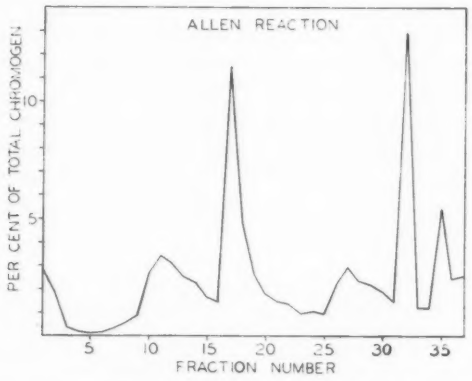

FIG. 3. Chromatographic separation of chromogen complex of neutral ether extract of cow urine with alumina as adsorbent and ethanol, benzene, and acetic acid as eluants.

of six different chromogens is seen, peak fractions being 1, 11, 17, 27, 32, and 35. Extracts of urine prepared by either acid or enzyme hydrolysis gave similar chromogen distribution on the chromatographic column.

Diurnal rhythm of chromogen excretion. Three lactating cows (two Holsteins and one Guernsey) were used to study possible diurnal variation in chromogen excretion over a 4-day period with 8-hour periods of urine collection (Table 3). Creatinine was also followed as a reference substance. The Allen reaction (470 mμ) was used for chromogen determination and the results are expressed as per cent of total daily output. From the table it will be seen that the period from 8 A.M. to 4 P.M. is that of peak chromogen and creatinine excretion, but the low period for chromogen excretion (12 P.M. to 8 A.M.) is not the low period for creatinine excretion (4 P.M. to 12 P.M.).

TABLE 3

The diurnal rhythm of excretion of urine chromogens and creatinine as a mean of 4 days

Period	Chromogen (*% of total*)	Creatinine (*g.*)	Creatinine (*% of total*)	Chromogen (*% of total*)	Creatinine (*g.*)	Creatinine (*% of total*)
		H677			H678	
12 P.M.–8 A.M.	25.07	4.22	37.00	23.04	3.83	34.68
8 A.M.–4 P.M.	45.06	3.89	34.14	48.76	4.42	40.01
4 P.M.–12 P.M.	29.87	3.29	28.86	28.20	2.80	25.31
Total	100.00	11.39	100.00	100.00	11.05	100.00
		G830			Means	
12 P.M.–8 A.M.	22.13	3.61	37.25	23.52	3.89	36.28
8 A.M.–4 P.M.	48.34	3.51	36.24	47.27	3.94	36.79
4 P.M.–12 P.M.	29.53	2.57	26.51	29.21	2.88	26.93
Total	100.0	9.69	100.00	100.00	10.71	100.00

DISCUSSION

It is remarkable that in cow urine a group of closely related chromogens is found which parallel in so many respects the 17-ketosteroids present in human urine, a fact that leads to confusion in the various studies with cow urine. According to Holtz *(10)* these chromogens are ionone derivatives, originating from carotenoids of dietary origin and presumably having no endocrine significance. Points of similarity between the two classes of substances are reflected in their place of occurrence, solubilities, conjugated state, methods adequate for hydrolysis, methods of purification and extraction, chromatographic behavior and color reactions.

It would seem that the work of most investigators *(2, 5, 7, 8, 14-17, 20, 21)* in studying the problem of 17-ketosteroids or neutral steroids in cattle urine has been confounded by the presence of these ionone chromogens. These ionone derivatives are present in cow's urine in relatively large quantities, and any adequate measurements of 17-ketosteroids in cattle urine must take specific account of these chromogens.

SUMMARY

Cow urine contains a group of chromogens which are apparently ionone derivatives of carotenoids of dietary origin. They are present in the conjugated state and may be hydrolyzed by either acids or B-glucuronidase. They are extractable with lipid solvents after hydrolysis. Specific absorption spectra were obtained with these chromogens by the use of the Zimmerman, Pincus, and Allen color reactions, but in each instance they differed from spectra obtained with 17-ketosteroids. These chromogens number at least six and react similarly to 17-ketosteroids on an alumina chromatographic column (benzene-ethanol system). The urinary excretion pattern of these chromogens displays a distinct diurnal rhythm. Studies to date on the measurement of 17-ketosteroids in cow urine have been confounded by the presence of these chromogens.

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THE EFFECT OF VARIOUS LEVELS OF DIETARY PROTEIN UPON THE VOLATILE FATTY ACIDS IN THE RUMEN OF THE DAIRY COW[1]

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Volatile fatty acids are the end products of the hydrolysis of complex carbohydrates produced by the bacterial population in the rumen. Nine cows were used in a feeding experiment to determine the influence of the amount of protein in the ration of a ruminant upon the level of volatile fatty acids formed. Experimental periods were 21 days in length. Increased amounts of volatile fatty acids except "higher acids" were observed as a result of high levels of protein intake. *Editor.*

The importance of volatile fatty acids in the nutrition of the ruminant has been thoroughly discussed *(3, 6)*. It has been recognized for some time that these acids are the end products of the hydrolysis of cellulose and other complex carbohydrates in ruminant rations by enzymes produced by the bacterial population in the rumen. Recently, Annison *(1)* and El-Shazly *(4)* have reported that the level of rumen volatile acids was increased when a purified source of protein was added to the ration. Similar reports from Gray *et al. (5)* and Chalmers and Synge *(2)* indicate that protein in purified rations may be a source of volatile fatty acids in the rumen. Lewis *(9)* has noted the production of volatile fatty acids from amino acids supplied to rumen microorganisms. In vitro studies by Sirotnak and coworkers *(12)* have indicated that rumen bacteria attack the amino acids, glutamic, aspartic, serine, arginine, cystine, and cysteine, with the resultant formation of volatile fatty acids.

The chemical reactions involved are poorly understood. There is a possibility that the Stickland *(14)* reaction is the basis for the formation of some of these acids, although Lewis *(9)* could not demonstrate this except in one case. They may also arise from the deamination of such amino acids as glycine, alanine, and aspartic.

It was deemed advisable to determine whether the amount of protein contained in a conventional ration for ruminants would influence the levels of volatile fatty acids present in the rumen. The following study was undertaken with this principal objective.

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EXPERIMENTAL PROCEDURE

Nine cows in late lactation, representing the Holstein, Guernsey, and Jersey breeds, were assigned to three replicates of a 3×3 Latin square. A basal ration of 1 lb. of U. S. No. 2 timothy hay and $1\frac{1}{2}$ lb. of well eared, good quality corn silage per each 100 lb. of body weight was fed each animal. Experimental treatments consisted of feeding 1 lb. per each 100 lb. of body weight of: *(a)* corn and cob meal (low protein), *(b)* a mixture of equal parts of soybean oil meal and corn and cob meal (medium protein) and *(c)* soybean oil meal (high protein). These treatments were calculated to supply: *(a)* approximately the minimum allowance of digestible protein recommended by Morrison's standards *(9)*, *(b)* twice the allowance and *(c)* four times this amount. The chemical composition of all feeds used is reported in Table 1.

TABLE 1

Chemical composition of feeds used in studying the effect of protein on volatile fatty acids in the rumen

Feeds	Water	Crude fat	Crude fiber	Crude protein	Ash	NFE
	(%)					
U. S. No. 2 timothy hay	6.7	1.9	34.5	5.9	3.7	47.3
Well cured corn silage	73.2	0.8	7.1	2.4	1.3	15.2
Corn and cob meal	8.6	3.0	8.2	9.2	1.9	69.1
Soybean oil meal	9.8	2.0	6.8	44.0	5.4	32.0

Experimental periods were 21 days in length. Samples of rumen contents were obtained orally 4 hours after feeding on the 12th, 13th, and 14th days of each period as well as on the 19th, 20th, and 21st days. The pH of each sample was determined immediately after drawing. The sample was then strained through cheesecloth, and an aliquot was preserved for storage by adding $2\frac{1}{2}\%$, by volume, of a saturated solution of mercuric chloride. Samples were stored at 5° C. previous to analysis.

The amounts of acetic, propionic, butyric, and valeric acids, and a mixture of longer chain acids, referred to as "higher acids," present in each sample were determined by the method reported by Keeney *(8)*.

Data were subjected to statistical analysis according to the methods reported by Snedecor *(13)*. Tests of significant differences between means were made by the technique of Scheffe *(11)*.

RESULTS AND DISCUSSION

The average amounts and proportions of volatile fatty acids found in the rumen liquid of cows fed three levels of protein are shown in Table 2.

Differences in the amount of acetic acid present were not statistically significant; however, a trend toward somewhat more acetic acid with the high protein rations was noted. The proportion of the total volatile fatty acids, represented by acetic acid, was decreased with increasing levels of protein in the ration. Differences between the three rations were significant at $P = < 0.01$.

TABLE 2

Average amounts and proportions of volatile fatty acids observed in the rumen liquid of cows fed three levels of protein

Fatty acid	Low protein				Medium protein				High protein			
	13 days feeding		20 days feeding		13 days feeding		20 days feeding		13 days feeding		20 days feeding	
	(*mM/l*)[a]	(*% of total acids*)	(*mM/l*)	(*% of total acids*)	(*mM/l*)	(*% of total acids*)	(*mM/l*)	(*% of total acids*)	(*mM/l*)	(*% of total acids*)	(*mM/l*)	(*% of total acids*)
Acetic	63.9 ±2.5[b]	61.6 ±1.0	65.4 ±2.1	62.3 ±0.5	63.8 ±3.6	58.8 ±1.1	69.8 ±1.5	61.3 ±0.6	72.5 ±2.3	58.7 ±0.3	67.8 ±3.0	58.1 ±1.0
Propionic	20.8 ±1.0	20.1 ±0.8	19.9 ±0.9	18.9 ±0.4	22.1 ±1.6	19.9 ±0.4	22.4 ±0.4	19.6 ±0.4	25.6 ±1.6	20.7 ±1.1	23.9 ±0.9	20.3 ±0.3
Butyric	14.7 ±0.9	14.1 ±0.4	14.8 ±0.8	14.0 ±0.3	17.2 ±1.1	15.6 ±0.4	16.7 ±0.5	14.7 ±0.3	19.2 ±1.0	15.4 ±0.4	17.9 ±0.8	15.3 ±0.3
Valeric	2.9 ±0.2	2.8 ±0.2	2.8 ±0.1	2.7 ±0.2	3.4 ±0.3	3.0 ±0.2	3.3 ±0.3	2.7 ±0.4	4.3 ±0.3	3.5 ±0.2	4.0 ±0.3	3.5 ±0.3
"Higher" acids	2.3 ±0.1	2.1 ±0.2	2.1 ±0.2	2.0 ±0.2	2.5 ±0.2	2.1 ±0.2	1.7 ±0.2	1.5 ±0.1	1.9 ±0.2	1.7 ±0.1	1.8 ±0.2	1.5 ±0.2
Total acids	104.6	——	105.0	——	109.0	——	113.9	——	123.5	——	115.4	——

[a] Millimoles per liter.

[b] Standard error of the mean.

The total amounts of propionic acid also were increased in the presence of extra protein with differences between the three rations significant at $P = < 0.01$. It is interesting to note that the proportion of propionic acid remained essentially the same for all three treatments.

Butyric acid levels were increased significantly ($P = < 0.01$) with both the medium and high protein rations as compared to low protein feeding. Differences between the medium and high protein rations were not statistically significant, but the data indicate somewhat larger amounts from the high protein ration. In contrast to the changes noted among the proportions of acetic acid, the relative amounts of butyric acid present were increased by high protein feeding. Differences between the two higher levels were not statistically significant, but both of these were significantly higher than the percentage observed for the low protein ration ($P = < 0.01$).

Statistically significant increases in the quantities of valeric acid present were observed for each change in protein intake ($P = < 0.01$). Differences in percentage of valeric acid were not statistically significant.

"Higher acids" were found to decline slightly in total amount as the level of protein intake increased. However, these differences were not statistically significant. The percentages of "higher acids" present with the medium and high protein rations were significantly less than that present on the low protein ration ($P = < 0.01$).

There was a definite increase in the total volatile fatty acids present in the rumen fluid in response to each increase in protein feeding ($P = < 0.05$). All of the acids studied were present in greater amounts after 13 days of feeding the high protein ration than at other times. The reason for this is not readily apparent.

It is difficult to estimate how closely the observed values reflect changes in actual production of the volatile fatty acids. Variations in the rate of absorption of these materials from the rumen under different conditions may tend to mask some of the differences which occur. However, it appears that the presence of additional protein in the ration caused an increase in the total amounts and a change in the proportion of certain of the volatile acids present in the rumen. From the work previously cited *(1, 2, 4, 5, 8)* it seems reasonable to assume that the protein may have served as a source of at least part of these acids.

Data from samples collected after 13 days of preliminary feeding showed considerably more variation among animals and among sampling days than that collected at 20 days. This fact is well demonstrated by the difference in size of the standard error of the mean obtained for the two periods. There were statistically significant differences ($P = < 0.05$) among the values observed for acetic, butyric, and "higher" acids after both 13 days and 20 days of feeding. The presence of considerably larger amounts of all fractions of volatile fatty acids after the feeding of the high protein for 13 days as compared to feeding for 20 days is striking. Whether or not this is due to changes in the rate of production of these acids or is accounted for by differing rates of metabolism or absorption cannot be determined at this time.

Comparisons of samples obtained on single days with the averages of samples taken on two and three days showed significant differences between these values ($P = < 0.01$) when obtained after 13 days of preliminary feeding. Similar comparisons made after 20 days of preliminary feeding indicated no statistical differences. It would appear, under the conditions of this experiment, that adjustment of the digestive tract to changes in the ration was not complete at 13 days and that a preliminary period longer than 2 weeks should be employed to assure the most accurate observations. Whether preliminary periods of more than 20 days may be necessary in some experiments cannot be established from this experiment. Jacobson *(7)* has reported biochemical changes continuing to occur in the rumen for several weeks after a change in the ration.

SUMMARY

Nine lactating cows were fed three levels of protein in a change-over design experiment. Samples of rumen liquor were obtained on three consecutive days, centered on 13 and 20 days of feeding each ration.

Increased amounts of all volatile fatty acids except "higher acids" were observed as a result of high levels of protein intake. Increasing the protein allowance caused a decrease in the percentage of acetic acid and "higher acids" and an increase in the percentage of butyric acid present in the total volatile fatty acids in the rumen.

Comparison of the data obtained after 13 days and 20 days of preliminary feeding indicated that adjustments within the rumen to changes in the ration were not complete after 2 weeks on a new ration.

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EFFECTS OF VARIOUS HAY-CONCENTRATE RATIOS ON NUTRIENT UTILIZATION AND PRODUCTION RESPONSES OF DAIRY COWS. I. RELATIONSHIPS AMONG FEEDING LEVEL, PREDICTED PRODUCING ABILITY, AND MILK PRODUCTION[1]

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Thirty-six Holsteins were divided into high, medium, and low producing groups in order to evaluate their efficiency for milk production. It was found, regardless of the ratio of concentrates to hay fed, that the inherent ability of cows to produce milk was more significant than the intensity of feeding. *Editor.*

There are many reports in the literature related to the nutrition, physiological response, and milk production of dairy cows fed various levels and combinations of roughages and concentrates. Mead and Goss *(9)* have studied ruminant digestion without roughage, whereas Sherwood and Dean *(14)* have employed alfalfa as the sole feed of dairy cattle over successive lactations. Huffman *(4)* has reviewed the effects of roughage quality and quantity in the dairy ration. The study reported by Martin *et al. (8)*, was designed to determine whether varying the rate of hay feeding within normal limits while maintaining a constant TDN intake affected the level of production or the well-being of dairy cows.

The experiment reported herein was designed to evaluate the relationships among and the effects upon milk production of: hay to concentrate ratio, producing ability of the cow, level of feeding, and changes in body weight. Other phases of the study, which include a digestion experiment, the excretion pattern of chromic oxide, evaluation of feeding standards, and evaluation of certain economic aspects, will be reported subsequently.

EXPERIMENTAL PROCEDURE

Design of experiment. Thirty-six Holstein cows from the Iowa State College herd entered the experiment from March, 1953, to September, 1954. The experiment consisted of a 14-day adjustment period, a 50-day preliminary period, and a

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182-day experimental period. During the adjustment period, which began at calving, each cow was placed in a stanchion or box stall and was fed a ration with a fixed hay:concentrate ratio (7 lb.:4 lb.) which was maintained throughout the preliminary period. The amounts of the feeds were increased within this constant hay:concentrate ratio to permit each animal to express its consumption capacity and productive ability. The production in the preliminary period was extrapolated to an 8-month lactation, and the production range of the Iowa State College Holstein herd provided the criterion for assigning the experimental animals to high, medium, and low producing groups. Throughout this work, the term "ability" is defined by and limited to that level of production, within a single lactation, attained over the preliminary period. The production ability ranges in term of fat-corrected milk (FCM) were "high"—10,500 lb. and above, "medium"—9,000 to 10,500 lb., and "low"—9,000 lb. and below.

TABLE 1
The experimental design

Level of feeding	Ability	Hay to concentrate ratio			
		H–75:C–25	H–55:C–45	H–35:C–65	H–15:C–85
		(Cow No.)			
High	High	2553	3157	2710	2982
	Medium	2392	2649	3529	2976
	Low	3632	3263	3160	3538
Medium	High	3266	2600	2378	3142
	Medium	3469	3444	2643	3493
	Low	3440	3597	3432	3516
Low	High	3272	3291	2963	3174
	Medium	3450	3483	3128	2606
	Low	3302	3294	3439	2159

The experimental design is shown in Table 1. Each of the four hay:concentrate energy ratios (75:25, 55:45, 35:65, and 15:85) was fed at a high, a medium, and a low level. One cow of high, one of medium, and one of low producing ability were randomly allotted to each of the 12 "hay:concentrate-feeding level" groups.

The arbitrary feeding levels were based upon the quantities of feed energy above body maintenance requirements necessary to produce 13,000, 11,000, and 9,000 lb. of FCM in a 243-day lactation period. Morrison's *(10)* higher net-energy allowances for maintenance and for the production of FCM were utilized in determining the energy required at each feeding level. The energy requirement for maintenance of each cow was determined from the average of the body weight measurements taken during the two pre-experimental periods. The feed energy required for milk production by each cow during the experimental period was computed and divided into 26 decreasing weekly portions according to the lactation curve of the College Holstein herd. Individual energy allowance for maintenance was held constant over this period.

Feeding and management. The concentrate mixture had the following composition in pounds: ground yellow corn, 400; crushed oats, 500; wheat bran, 200;

soybean oil meal, 200; linseed oil meal, 100; steamed bonemeal, 28; and salt, 14. Five grams of a trace mineral mixture were added daily to the concentrate allowance of each cow. Second-cutting alfalfa hay was the primary roughage fed. Aliquots of concentrates and of hay fed each day were composited and were analyzed periodically. The concentrate mixture had an average analysis in per cent of: fiber, 7.5; protein, 17.1; ether extract, 2.5; ash, 5.6; nitrogen-free extract, 55.2; and moisture, 12.0. For hay the average percentage values were: fiber, 28.0; protein, 13.7; ether extract, 1.5; ash, 6.7; nitrogen-free extract, 39.3; and moisture, 10.9. All feeding was based upon the estimated net energy (ENE) standard and the energy values of the feeds were calculated from the proximate feed analyses, applying digestibility coefficients taken from Morrison *(10)*. Because of the nature and amounts of rations employed, concentrates were fed four times daily, as was hay when offered in amounts above 20 lb.

Throughout the entire experiment body weights were taken at 4-day intervals, milk weights were recorded at each milking (twice daily), and milk-fat analyses were obtained weekly. Milk production data were converted to FCM values. The experimental period was concluded 8 months postpartum at which time each cow was returned to normal herd ration and management. FCM data for the post-experimental period were calculated from daily milk weights and monthly milk-fat analyses until the cows were dry or had lactated 4 months.

The following model for analysis of variance was used extensively in this work:

Source of variance	*d/f*
Ration (hay: concentrate ratio)	3
Level (feeding level)	2
Ability (prelim. period production)	2
Ration × level (R × L)	6
Ration × ability (R × A)	6
Ability × level (A × L)	4
Remainder	12

The remainder was used for tests of significance.

RESULTS

Feed input and milk production. The distinct distribution of the high, medium, and low ability cows in each hay:concentrate-feeding level group as based on preliminary period productivity was implemented, with minor exceptions. However, there was some overlapping in productivity among the groups. For example, a high ability cow in one group may have had a production level equivalent to that of a medium ability cow in another group. The fixed ratio of hay:concentrate feeding of 7 lb.:4 lb. (1.75:1), established for the preliminary period, was closely approached, since the mean ratio for all cows was 1.67:1.

The chief difficulties encountered during the experimental period were *(a)* inability of those cows placed at high feeding, high hay levels to consume the allotted energy, *(b)* occasional digestive disturbances among cows on the high concentrate ratios, and *(c)* the strong and regular depression of the higher levels of hay consumption by environmental temperatures of 80° F. and above.

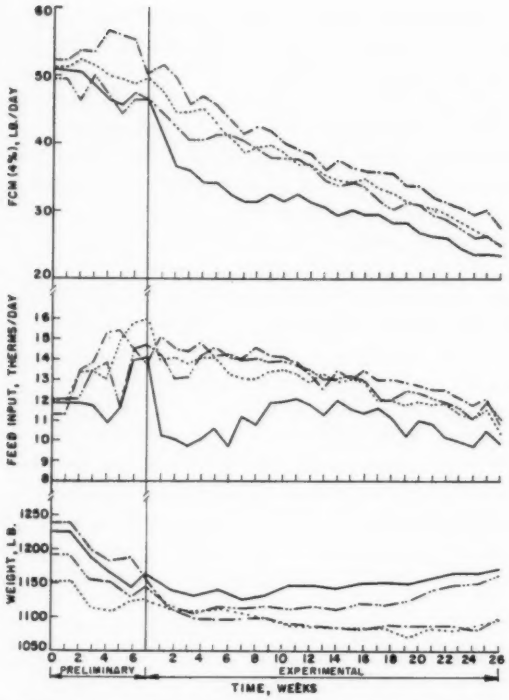

FIG. 1. Mean milk production, feed inputs (expressed as therms above maintenance), and body weight of each group of nine cows at each hay to concentrate ratio. 75% hay:25% conc. = ————, 55% hay:45% conc. = ... , 35% hay:65% conc. = - - - - - , 15% hay:85% conc. = - . - . - .

Figure 1 presents milk production, energy input (in therms) above maintenance and weight changes over the preliminary and experimental periods. In this and the similar graphs which follow, each pound of FCM is scaled in terms of its energy content (0.3 therm) so that the graphs for FCM and feed input are directly comparable. These data indicated that, across the four hay:concentrate ratios, milk production closely followed the ENE inputs of feed above maintenance requirements. The depressed milk production and feed input curves of the cows at the 75:25 (hay:concentrate) ratio over the experimental period indicated their inability to consume the required energy largely in the form of hay.

The nine cows fed the highest concentrate rations consistently produced more milk than the groups at the 55:45 and 35:65 (hay:concentrate) ratios. However, the total energy inputs above maintenance for the three groups of nine cows each at the 55:45, 35:65, and 15:85 ratios were, respectively, 20,808, 21,280, and 21,874 therms. The increased milk production at the highest concentrate ratio was not explained entirely by the differences in the input of therms, and further explanation was sought from data in the preliminary period.

The analysis of variance of milk production in the preliminary period revealed that not only was the portion of variability due to ability very high (it was on this basis that the animals were grouped) but the variance among ration groups was statistically significant ($P \leq 0.05$). This is seen in Figure 1 from the milk production curves of the preliminary period where the 15:85 (hay:concentrate) ration curve is considerably higher than those of the other three groups. The totals of milk production of the four groups of cows, from the high to the low hay feeding ratios, were, respectively, 21,622; 22,656; 21,193, and 24,251 lb. during the preliminary period.

The milk production in the experimental period was adjusted by covariance for the differences in production in the preliminary period. Before the adjustment, the average milk production from the high hay to the low hay rations were, respectively, 5,482; 6,494; 6,322, and 6,936 lb. The means adjusted for the differences in the production during the preliminary period were, respectively, 5,570; 6,469; 6,456, and 6,737 lb. The level of production in the preliminary period was related to the level of production in the subsequent experimental period. The correlation between production in the preliminary period of each cow with that in the experimental period, regardless of the ration and the level of feeding to which the cow was allotted, was 0.63. When ability (A) and its interaction terms in the analysis of variance model ($A \times R$, $A \times L$, $R \times L \times A$) were considered alone, the correlation rose to 0.90. This indicated that the establishment of conditions before and during early lactation appeared to influence the course of subsequent production, regardless of the treatment to which the animal was subjected.

Body weight changes. Mean weight changes during the experimental period varied among ration groups (Figure 1). Animals at the highest concentrate level (which produced the most milk) showed the greatest weight losses. Thus differences in experimental period production among ration groups may be attributable in part to differences in weight changes. Apparently there was a greater stimulus for milk production among certain groups even though weight loss was necessary to accomplish the increased production.

The largest and most rapid changes in body weight, actual flesh and/or body fill, took place over the first 4 to 5 weeks of the experimental period. After this early phase of the experimental period the cows maintained body weight or changed at a slow and rather uniform rate.

The total changes over the entire experimental period and the changes from the end of the initial 4 weeks of the experimental period to its conclusion were analyzed statistically. When the entire experimental period was considered, the effects of ration, feeding level, and ability upon body weight changes either approached statistical significance or were significant at $P \leq 0.05$. When the last 22 weeks were considered, these effects were reduced. The residual component of variance increased from 50% of the total variance in the first case to 74% in the second and from the first to the second case, the components for ration, feeding level, and ability decreased (Table 2). Thus, body weight changes after

TABLE 2

Division of the variance in weight changes, energy intake, and milk production during the experimental period

Source of variance	Component of variance	Wt. change		ENE for prod. above maint. for exptl. period	Milk prod. during exptl. period
		0–26 wk.	4–26 wk.		
		(%)	(%)	(%)	(%)
Ration	R	9	6	17	27
Level	L	15	0	55	2
Ability	A	15	0	2	31
R × L	RL	2	14	11	10
R × A	RA	9	6	3	10
A × L	AL	0	0	0	0
R × L × A	E	50	74	12	20

the first 4 to 5 weeks of the experimental period were largely independent of the ration fed, the level of feeding, and the ability of the animals.

The relationships among feeding level, producing ability, and milk production. Differences in the arbitrary levels of feeding apparently were not sufficiently extreme to overcome the productive ability classification of the cows based upon the preliminary period. Figure 2 illustrates the tendency of the animals, though

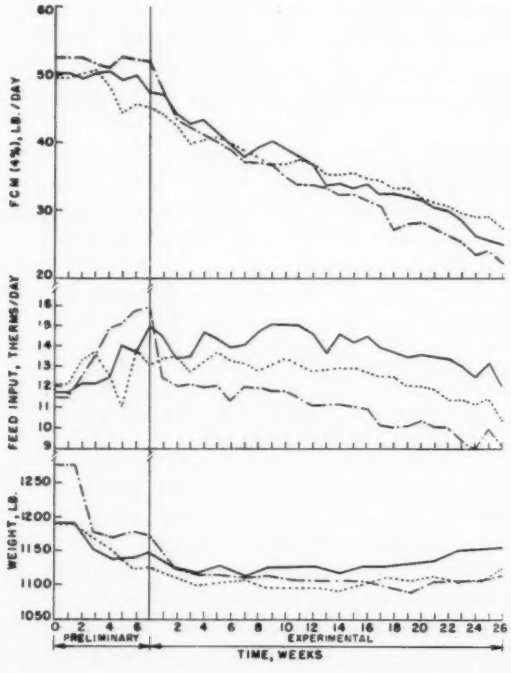

FIG. 2. Mean milk production, feed input and body weight changes of each group of 12 cows at each of the three feeding levels. High = _____, Med. = ... , Low = - - - - -

fed at different levels, to move toward a general milk output level whereas weight changes in either direction remained relatively small. Many cows of high producing ability placed at the low and medium levels of feeding were able to produce milk at levels equal to those of the cows at medium and high levels of feeding. Figure 3 presents this phenomenon as it was observed among the cows at the lowest feeding level. Although similar feed inputs in therms were maintained at the low feeding level for four cows each of high, medium, and low producing ability across the four hay:concentrate ratios, the milk production became sharply separated on the basis of ability. These differences in production remained distinct after largest weight changes had ceased and a relative stabilization of weight had been achieved.

When the consumption of ENE available for milk production was considered, the tests for the effects of the ration (R) and the level of feeding (L) were sta-

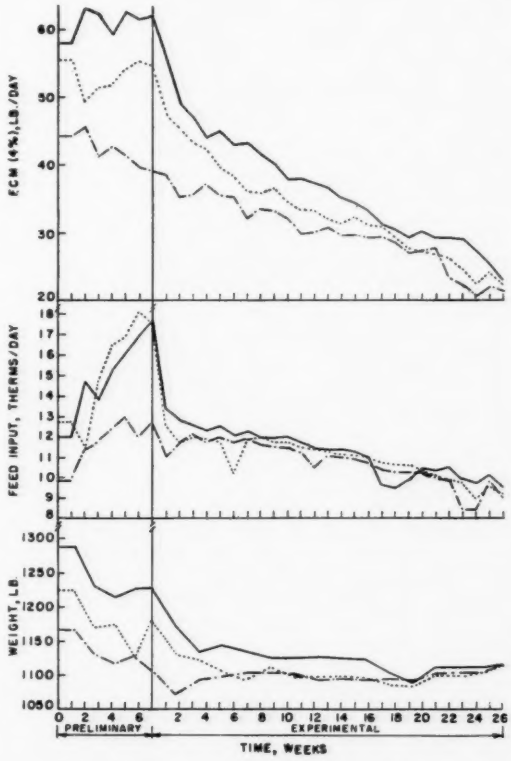

FIG. 3. Effect of producing ability on milk production and on weight changes of cows at the low level of feeding across the four hay to concentrate feeding ratios. (Feed inputs are expressed as therms above maintenance and each line is an average of four cows.) High = ————, Med. = ..., Low = - - - -

tistically significant ($P \leq 0.01$). The differences in the effects of the ration were caused by the inability of the cows to consume equally well the required energy over the four hay : concentrate ratios. The differences in the effect of the feeding level were consistent with the three levels within the experimental design.

When milk production for the experimental period was considered, the effects of the differences in feeding levels were not significant. On the other hand, the effects of the ration (R) and ability (A) of the cows upon milk production were statistically significant ($P \leq 0.01$). The component of variance (Table 2) for the level of feeding (L) was 55% when ENE available for production was considered, whereas it was reduced to 2% when milk production was considered. The opposite result was obtained with the component for ability (A), which rose from but 2% in the first case to 31% in the second. The average production per cow based on the high, medium, and low feeding levels were, respectively, 6,453, 6,453, and 6,021 lb., whereas those based on high, medium, and low ability were, respectively, 6,844, 6,431, and 5,651.

Post-experimental period. Data were collected during this period to determine whether the differences in the preliminary period production would be reflected in an advanced stage of lactation at the conclusion of the experimental period when the cows were returned to full feed and normal herd management. Feed consumption data were not obtained; hence, the data are not critical in nature but are presented for the appearance of trends.

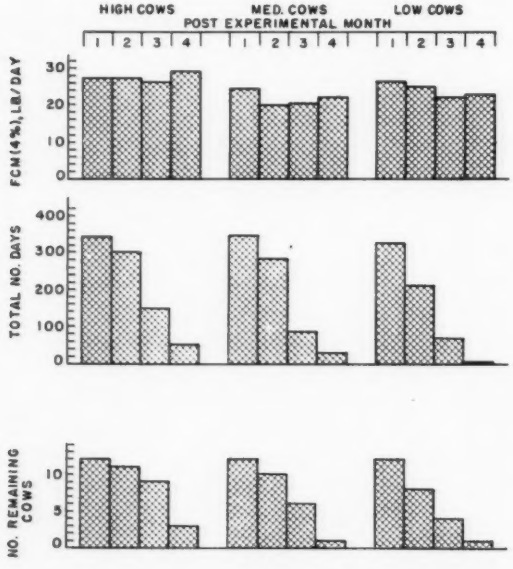

FIG. 4. Post-experimental production and persistency (absolute rank based on preliminary period production).

In the experimental design it was not possible to insure that each animal in a particular ability group had a higher preliminary production than each animal in a lower ability group. As a result, a certain amount of overlapping occurred among the ability levels. To eliminate this effect, the results in Figure 4 represent the cows as grouped into three absolute ability groups of 12 cows each, ranked from the highest to the lowest on the basis of preliminary period production. The results for each month are classified vertically into three segments: average pounds FCM per cow per day, total number of days milked, and the number of remaining cows. When the units of data are combined, the histograms show that the high cow group, based on the classification described above, produced milk at a higher level, over a greater total number of days and retained more cows for a longer period of time than did either the medium or low cow groups. Over this entire period with the cows classified as described, the total milk production for high, medium, and low cow groups were, respectively, 22,870, 16,488, and 15,570 lb. These data indicated the trend for the higher producers in the preliminary period to be more productive and persistent at the later stages of lactation.

DISCUSSION

Ability, as defined in this work, appeared to have been the major factor influencing the results obtained. How high ability cows produced more milk than medium ability cows receiving approximately equal amounts of feed energy above maintenance (Figure 3) is not clear. To illustrate, data for seven pairs of cows fairly comparable in body weight, age, type of stall, and time on experiment were tabulated (Table 3). These data encompassed the last 22 weeks of the experimental period, during which the cows were well adjusted to the various rations and their body weights were relatively stable. On an individual pair or an averaged pair basis, the large differences in milk production were not entirely explained by differences in feed input above maintenance (in this case calculated on the basis of the average weight over the experimental period), body weight changes, and dry matter digestibilities. (The digestibility data will be considered extensively in a subsequent paper.) When these data were summarized and expressed on an individual cow basis, the high ability as compared to the medium ability cow consumed 53 more therms, lost 16 lb. more, and was 34 lb. heavier in body weight. The average dry matter digestibility of the rations was higher for the medium ability cows. When these differences were converted to a milk equivalent basis, they represented a total of 440 lb. of FCM, whereas the average difference in production was 814 lb. The remaining 374 lb. milk per cow apparently were not associated with the measurements made in this work.

A few high and medium cow pairs in Table 3 deserve individual consideration. On equal feed inputs above maintenance cow 3174 produced 909 lb. more milk, lost 60 lb. less weight and had a slightly lower digestibility than did cow 2606. Even with the small difference in average body weight in favor of cow 3174 removed, the large remaining difference remained unexplained. Not so

TABLE 3

Feed input, milk output, and related measurements for seven pairs of high and medium ability cows

Ability	Cow	Hay: conc. ratio	Feeding level	Feed input above maint.[a]	FCM output[a]	Av. body weight	Weight change[a]	Dry matter dig.	Daily FCM first month post exp. per.
				(therms)	*(lb.)*	*(lb.)*	*(lb.)*	*(%)*	*(lb.)*
High	3174	15:85	Low	1755	6126	1091	−31	62.9	39.5
Medium	2606	15:85	Low	1747	5217	1137	−91	65.8	26.0
High	2982	15:85	High	2429	6612	1223	−21	58.3	29.2
Medium	2976	15:85	High	2391	5900	1195	−38	58.5	24.7
High	3157	55:45	High	2314	6482	1204	−48	53.3	25.5
Low[b]	3263	55:45	High	2276	5374	1121	− 7	60.7	19.0
High	2600	55:45	Medium	2049	6377	1373	−31	57.5	35.2
Medium	3444	55:45	Medium	1883	5056	1016	26	64.4	19.7
High	2963	35:65	Low	1706	5353	1075	−21	56.0	27.0
Medium	3483	55:45	Low	1672	4783	1027	− 5	56.1	22.9
High	3266	75:25	Medium	1848	5584	994	7	48.3	45.9
Medium	3469	75:25	Medium	1868	5196	1334	57	55.6	30.4
High	3272	75:25	Low	1665	4703	1174	−53	58.2	24.7
Medium	3450	75:25	Low	1561	4012	1064	−24	56.1	18.9
Average									
High	——	——	——	1967	5891	1162	−28	56.4	32.4
Medium	——	——	——	1914	5077	1128	−12	59.6	23.1

[a] The period from the end of the first 4 weeks to the end of the experimental period.

[b] Preliminary period production of cow 3263 equivalent to that of medium cow 2649 at the same hay: concentrate-feeding level position.

radical but similar results were obtained with the high cow 2982, which outproduced the medium cow 2976 by 712 lb. The high cow 3266, on experiment from June to December, produced 388 lb. more milk and consumed less feed than did the medium cow 3469, on experiment from November to May. After the body gain of 3469 was equated with this difference in milk production, the lower intake of therms by cow 3266 still indicated her ability to produce more milk on a fixed dietary regime than cow 3469, despite the handicaps of smaller body size and the strongly depressive effect of hot weather upon high hay consumption. The data presented for the high hay ration offered to high cow 3272, on experiment over a summer season, and to the medium cow 3450, on experiment during the winter and spring, should also be considered in this light. Besides these individual pair comparisons, all the high ability cows considered here responded with greater daily milk production (32.4 lb.) during the first month post-experiment than the medium cows (23.1 lb.).

As this experiment was not designed to secure the many physical measurements needed to explain the unanticipated results, their partial clarification was sought from the following considerations: changes in the solids-not-fat (SNF), compensatory changes in maintenance requirements, and genetic effects.

Riddet *et al. (11, 12)* and Lutz *(6)* have shown that underfeeding of lactating cows caused a decline in the SNF content of the milk from 0.3 to 0.6%, which involved mainly the total protein fraction. These findings can be applied to the cows at the low level of feeding in the present study. It seemed reasonable that such a condition would be operative upon both the high and medium ability cows at the low level of feeding and would cause a similar degree of depression with both. However, if the depression of the SNF content occurred only with the high ability cows then part of the apparent production differences could be explained. Depression in the SNF content cannot explain the observed differences in the well fed animals, unless there was a definite depressive effect of ability upon the SNF content of the milk. Bonnier *et al. (2)* in their work with monozygous twin cattle have found that, in the estimation of the influence of heredity (ability) and environment (feeding level) upon the percentage of fat, protein, and lactose in milk, heredity played a much more prominent role than did differences in feeding intensity.

Ritzman and Benedict *(13)* reported that the individual and the breed were more important than character of the feed in determining the amount of energy required for maintenance. Blaxter *(1)* reported a variation of ±15 to 25% among cows in net energy requirements for maintenance. Superficially, it would appear that this provides an answer to the observed differences in production. However Washburn *et al. (15)* proposed that there should be an incidental higher heat production during lactation as a result of: *(a)* a greater nutrient turnover, *(b)* an increased level of endocrine activity, and *(c)* a higher maintenance cost of the hypertrophied mammary gland and associated organs. His studies showed that heat production in the nonfasting animal is related to milk production. Thus a high ability cow, producing at a high rate, should not have a greater economy of maintenance and associated requirements than the lower producing medium ability cow.

Bonnier *et al. (2)* have provided parallel evidence to support the contention that the genetic effect (ability) offered the most plausible, but by no means a complete, explanation of the results observed in the present work. In their growth and lactation studies with monozygous cattle twins *(2)*, one twin of each pair was fed at a low level and the other at a high level. It was observed that genetic effect usually outweighed the effects of differences in feeding intensity, even when these differences were large. This is an accord with the results of the present investigation. The physiological factors involved, however, are not clear, and further work undoubtedly will be required before the numerous factors in the milk production–ability complex can be unravelled. Milk yield responses, similar to those in this research, which have not received full explanation despite the measurement of a greater number of factors influencing production, have been reported recently by Brumby and Hancock *(3)*. These workers found that the subcutaneous injection of growth hormone to lactating identical-twin cattle induced a marked increase in milk and butterfat production concomitant with an apparent increase in the efficiency of production in the treated as compared to the controls. The authors offer the explanation that these results may be due to the known ability of growth hormone to enhance fat catabolism and protein production. In the present study a similar tissue change may have occurred in the treated cattle, thus providing endogenous energy. This study with growth hormone would support the view that the differences in ability, as measured in this current work, were largely brought about and controlled by the hormone complex of lactation.

The following may provide further evidence in support of the above view. Madden *et al. (7)* found that the genetic correlations between cumulative part and whole records had values approaching unity, and Leroy *(5)* provided data indicating that milk yield at any time during the lactation was definitely related to initial and maximum yields. These results have received further verification in this current work, despite some of the severe experimental conditions employed, and, together, these data strongly indicate that the conditions regulating subsequent milk production appear to be determined at the outset of lactation. Production will then attempt to proceed throughout the lactation in accord with the initial regulatory conditions, despite the induced environmental effects. This regulation continued to exert its effect in the post-experimental period, as is indicated by the differential production responses of the high, medium, and low ability cows.

The use of a constant ratio (in this case 7 lb. hay:4 lb. concentrates) in the preliminary period, since it is not tied to the particular and changing milk production of each animal, can be used to view feed consumption and production of animals from a common basis. This system of feeding during the first 60 to 90 days of lactation deserves further study. It may serve as an indication of the efficiency of animals and, as early production is highly correlated with subsequent production, this modified management as opposed to feeding to individual production may give a more valid estimate of the actual producing ability of an animal.

SUMMARY

Hay and concentrates in four ratios (75:25, 55:45, 35:65, 15:85) were fed to lactating cows for 182 days, starting 64 days after parturition, in isodynamic amounts above individual maintenance requirements at each of three feeding levels (high, medium, low). Thirty-six Holsteins were divided into high-, medium-, and low-producing groups, and three cows, one of each group, were fed at each of the 12 hay:concentrate-feeding level positions. For each cow the total feed energy requirement for production was predetermined and partitioned into decreasing weekly quantities. Feeding was based on net-energy recommendations for maintenance and production.

The differences in the ENE available for milk production at the three feeding levels were much greater than the corresponding differences in FCM production, which indicated that the ability of the animals exerted a greater influence upon production than did the intensity of feeding. Production in the preliminary period was highly correlated with that in the experimental period. The production response to full feed during the post-experimental period indicated a relation to the magnitude of production in the preliminary period.

Weight changes during the initial 5 weeks of the experimental period were closely related to diet but thereafter, particularly at the lower feeding levels, were largely independent of the ration.

ACKNOWLEDGMENT

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SERICEA, *L. CUNEATA*, WITH AND WITHOUT A NITROGEN SUPPLEMENT AS A SOURCE OF ROUGHAGE FOR LACTATING DAIRY COWS

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Lactating cows utilized their TDN more efficiently for milk production on alfalfa hay than they did on sericea hay which probably contained a depressant for milk production. *Editor.*

Generally, high yields of milk or meat have not been obtained when farm animals were fed sericea as the only roughage *(9, 13, 19, 23, 24)*. In a recent study, however, calves fed a mixture of chopped sericea and cottonseed meal, with a digestible protein content of approximately 10.5%, made significantly greater weight gains than those that received sericea without cottonseed meal *(10)*.

Digestion studies *(8, 10, 19)*, have shown that sericea is relatively low in digestible dry matter and in TDN; at least two studies would indicate that energy intake is not the primary deficiency of sericea for dairy animals *(8, 9)*. An apparent deficiency of some sericea forage is the ash content *(10, 11, 19)*, which was emphasized by the craving of calves for mineral salts *(22)* when sericea was the only roughage fed. Nevertheless, the calves receiving sericea with and without cottonseed meal supplementation had similar levels of serum inorganic P *(10)*. Thus, the results of the calf-growth study suggested: *(a)* that the primary deficiency of sericea, at least for growing calves, is the digestible protein content, which averages approximately 4.0% on the hay basis *(8, 10, 19)* and *(b)* that by correcting the insufficiency of digestible protein and providing TDN according to the recommended allowances *(20)*, lactating cows could be expected to produce milk at a rate similar to that obtained when a high quality forage is fed.

The good growth response of calves from supplementing sericea with cottonseed meal prompted an investigation to determine the response of lactating dairy cows fed sericea with and without urea as a protein substitute to provide a digestible protein equivalent similar to that of the digestible protein in alfalfa hay.

EXPERIMENTAL PROCEDURE

Fifteen lactating dairy cows that had passed their peak in milk production were assigned to the basal diet, described later, during a 2-week standardization period. On completion of the standardization period, the cows were assigned at random to the basal diet or one of the two test diets for the experimental period of 4 weeks duration. A description of the basal and test diets follows:

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(a) basal—alfalfa hay plus concentrate; *(b)* test—(S) sericea hay plus concentrate; and (SU) sericea hay supplemented with 262-urea at the rate of 11.7 g. per pound of hay, plus concentrate. It has been reported that urea has a digestible coefficient of approximately 70% as a protein substitute *(6, 16)*; thus, the 11.7 g. of urea added to the sericea hay was calculated to supply in the sericea-urea mixture a "digestible protein equivalent" of 10.5%, which is similar to the digestible protein content of good quality alfalfa hay *(20)*. The ingredients in the concentrate mixture and their percentage ratios were: corn, 39; citrus pulp, 38; cottonseed meal (41% protein grade), 20; steamed bone meal, 2; and salt, 1.

During the standardization period, a limit was placed on the hay intake of a few of the cows in order to have more than one cow within a given range; however, most of the cows received all of the hay they would eat, which supplied between 42.0 and 72.7% of the TDN fed. The quantity of hay fed during the experimental period was placed at the levels of average daily intakes during the standardization period. Nutrient requirements were estimated from the level of milk production during an interim of 9 to 14 days preceding the standardization period and from body weight at the beginning of the standardization period according to the recommended allowances *(20)*. The TDN fed during the standardization period was calculated to supply requirements for maintenance plus 100 to 112.5% of the TDN needed for milk production at preliminary period levels of production. Because of changes in level of milk yield of some cows during the standardization period, the TDN fed supplied between 86 and 135% of the recommended allowance *(20)*. The average intakes of TDN during the experimental period, as a percentage of requirements for production at the standardization period levels by dietary groups were: alfalfa, 108.2; sericea, 106.4; and sericea-urea, 107.8. The variations between these levels were not significant ($P \leq 0.05$). Differences between the total nutrient intakes and the nutrients fed as hay were supplied in the form of the concentrate mixture previously described.

The feeding procedure during the experimental period was similar to that followed during the standardization period, except that different forages were fed to cows receiving the test diets. The sericea forage in diets S and SU was fed at a rate that would provide the cows with approximately the same forage TDN as that supplied by alfalfa during the standardization period. Nutrients equal to the amounts in refused forages were supplied by feeding extra quantities of concentrates to maintain as nearly as feasible an iso-caloric intake throughout the standardization and the experimental periods.

Three of the five cows on each treatment were housed and fed individually in box stalls and the other two cows on each treatment were housed and fed individually in stanchions. The concentrate and the forages, which were chopped to approximately 3-in. lengths, were fed twice daily and the quantities consumed were recorded. Water was available at all times to cows in individual box stalls, whereas those housed in stanchions were watered twice daily. The individual box stalls and stanchions were bedded with pine shavings. Manure

was removed and clean shavings were added daily. The experimental cows were turned into a vegetation-free paddock to exercise during a period of 1 to 4 hours daily. Normally this was between 8:30 A.M. and 1:00 P.M.

Cows on all dietary treatments were machine-milked twice daily. Frequent checks were made to insure that milking was complete. The quantity of milk produced at each milking was determined and recorded. Butterfat content of the milk from individual cows was estimated by the Babcock method *(1)* on a 2-day composite at 2-week intervals; i.e., at the beginning and at the end of the experiment and once while the experiment was in progress. All milk yields were converted to FCM *(4)* for comparison.

All cows used on this experiment were weighed on the first three days of the standardization period, on the last three days of the standardization period, and on three successive days at the end of the experiment.

Initial occurrence and duration of abnormalities in the health of the cows were recorded on individual barn sheets.

RESULTS AND DISCUSSION

The digestibility of the experimental feeds as determined by conventional digestion trials are shown in Table 1. The TDN content of the alfalfa hay and some of the sericea hay was relatively high *(20)*. The comparatively high TDN content of the alfalfa hay seems to be in keeping with the leafiness of the hay. The TDN content of the sericea hay fed at the first of the experiment was higher than that fed during the later part of the study as determined by a digestion trial that preceded and one that followed the feeding study with the lactating cows. Sericea fed in both digestion trials was relatively high in digestible protein content, that fed during the latter trial providing the highest level. Since the digestible protein content of the sericea fed in the second trial was unusually high, a recheck was made on the crude protein content of the feed and the feces. The recheck confirmed the original analyses. Nitrogen content of the feed and feces was measured by the micro-kjeldahl method *(1)* and multiplied by 6.25 to give the crude protein contents.

The average pre-experimental milk production of cows assigned to alfalfa and sericea-urea groups was similar and slightly higher than that of cows

TABLE 1

Total digestible nutrient, digestible protein and dry matter content, of experimental feeds on the air-dry basis[a]

Forage	DM	TDN	DP
	(%)	(%)	(%)
Digestion Trial I			
Alfalfa hay	92.95	55.30	9.20
Sericea hay	29.00	51.01	4.34
Concentrate	89.90	74.40	10.40
Digestion Trial II			
Sericea hay	89.50	45.31	6.96
Sericea hay, av. 2 trials	90.75	48.16	5.65

[a] DM is dry matter, TDN is total digestible nutrients, and DP is digestible protein.

assigned to the sericea group. This slight difference in the initial production would be expected to affect the level of production but not the persistence of milk yield during the experimental period. Therefore, milk production for the experimental period was adjusted by covariance *(21)* to take into account the difference in initial levels of production of individual cows. The quantities of FCM produced during the standardization and the experimental periods and the adjusted production for the experimental periods are given in Table 2.

TABLE 2

Mean daily FCM yields, by periods, by dietary groups

Dietary group	FCM by periods		Adjusted experimental production FCM
	Standardization	Experimental	
	(lb.)	*(lb.)*	*(lb.)*
Alfalfa hay	30.70	28.74	27.87
Sericea hay	28.58	21.60	22.72
Sericea hay–urea	30.02	22.70	22.49
L.S.D., P = 0.01	——	——	4.20

During the experimental period, the mean adjusted FCM yields of cows fed sericea and of those fed sericea-urea were similar but were significantly less than the average yields of cows fed alfalfa hay as the source of roughage. The lower milk yields of cows fed sericea than those fed alfalfa are in agreement with previous reports *(9, 13, 24)* of studies in which nutrient intakes were not controlled. The 6.6% decrease in production during the experimental period by cows in the alfalfa group was within the range of the 6 to 8% decline expected *(22)*; whereas, the 25.98 and 24.82% decreases in FCM of cows in the sericea and sericea-urea groups, respectively, were excessive. Decreases in milk yield of cows in this study cannot be accounted for by differences in TDN intake, since the levels of TDN consumed by all groups were similar and averaged 6.4 to 8.2% in excess of requirements. Also, the average apparent digestible protein intake of each cow in all groups was adequate to sustain production of milk at the standardization period level. The adequacy of the digestible protein supplied by the sericea hay for the experimental period was unexpected and resulted from the 5.65% apparent digestible protein content of the sericea hay fed, which is relatively high *(8, 10, 19)*. Because of the variability in the digestible protein content of the sericea hay, it is possible that cows fed diet S received inadequate quantities of digestible protein at some stages of the experimental period. If this were true, the urea in diet SU should have ameliorated the condition, yet there was no apparent lactation response to urea feeding.

Since the primary objective in feeding dairy cattle is to obtain the greatest possible efficiency in converting consumed nutrients into milk, the gross efficiency of milk production was computed from the formula described by Morrison *(20)*, i.e., Gross Efficiency = 340 Cal. × lb. FCM ÷ 1814 Cal. × lb. TDN. In order to account for individual variation, the gross efficiency during the experimental periods was adjusted by using the gross efficiency during the standardization

period as a covariable. Possibly it should be re-emphasized that during the standardization period all cows received the same ration. The gross efficiency of milk production during the standardization period and during the experimental period and the adjusted gross efficiency of milk production during the experimental period are shown in Table 3. The gross efficiency of milk produc-

TABLE 3

Gross efficiency of milk production, by periods, as related to diets

Diet group	Standardization period	Experimental period	Experimental period, adjusted
	(%)	(%)	(%)
Alfalfa	31.1	28.7	28.9
Sericea	30.9	24.1	24.4
Sericea–urea	32.0	24.1	23.6
L.S.D., P = 0.01	—	—	4.1

tion by cows fed alfalfa was greater ($P \leq 0.01$) than that of cows fed sericea and sericea-urea, respectively.

The body weight data during the experiment were examined to determine whether the decreases in milk production of cows on diets S and SU were reflected in weight changes. Cows fed alfalfa gained an average of 0.92 lb. in body weight daily, which was similar to the 0.87 lb. gained by cows fed sericea but significantly greater than the 0.07 lb. loss in weight of cows fed sericea-urea.

The combination of excessive decreases in milk production and varied changes in body weight by cows fed sericea and sericea-urea suggested differences in utilization of the TDN consumed. A study of the forage intake data revealed that cows fed alfalfa hay consumed an average of 61.0% of their TDN in the form of hay, whereas cows fed sericea and those fed sericea-urea consumed 56.6 and 51.3%, respectively, of their TDN as hay. Furthermore, there appeared to be a positive correlation between the percentage of TDN supplied as hay and the percentage decrease in milk production. A test of this relationship (Figure 1) revealed that there was a significant decline in milk production with each increase in the percentage of the TDN supplied by the hays and the decrease for sericea-fed cows started at a lower level of intake than for alfalfa-fed cows. Thus, when cows are fed sericea hay it appears that the average monthly rate of decline in milk production is a function of the TDN requirements supplied by sericea and may be expressed as: $y = (x\text{-}a)(b)$ when a is the maximum percentage of TDN from sericea hay that may be fed before any decline in production would be expected, b is the regression coefficient, x is the per cent of the TDN supplied by hay and y is the expected rate of decrease in production of FCM. In this study, the coefficients for sericea and sericea-urea were 19.2% and 15.6%, respectively, and the a coefficient for alfalfa was 51.6%. The difference in a coefficients between diets were statistically significant. There appear to be three logical means for accounting for the difference in the rates of decline of FCM by cows when fed the same percentage of their TDN requirements from different hays. They are: *(a)* differences in the net energy values of

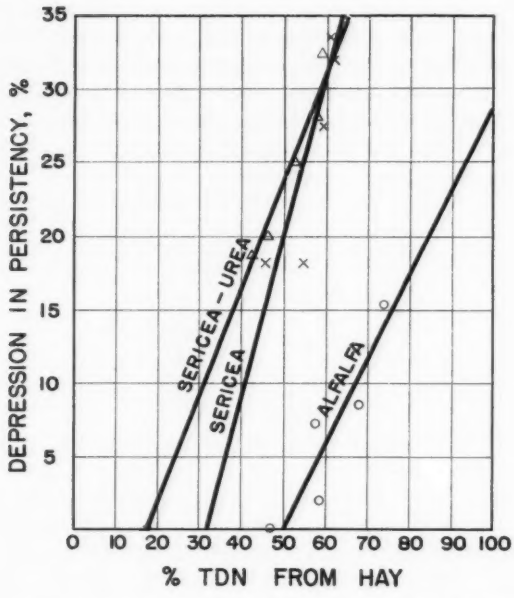

FIG. 1. Relationship between percentage TDN supplied by hay and percentage depression in persistency of milk yield per month.

the hays, *(b)* the presence of a lactation depressant in the sericea hay, and *(c)* the presence of some constituent in sericea hay that made digestible protein unavailable to the cows.

The variable net energy–TDN ratio concept has support from data in the literature which show that the digestible nutrient content of feedstuffs may not be a good measure of their productive value *(17)*. According to Morrison's estimates of net energy *(20)*, the alfalfa hay should have provided approximately 43 therms of net energy per 100 lb., whereas the sericea forage possibly provided as little as 30 to 36 therms, using low quality roughages including carpetgrass hay, cottonseed hulls, and corn fodder with ears removed as the basis for the latter values. Since the average crude fiber content of the sericea hay was 29.2, which is similar to that of average quality alfalfa hay *(20)*, whereas the 26.4% crude fiber in the alfalfa hay was low, there is a possibility that equal amounts of TDN from the two hays were not equal in net energy. Nevertheless, it would appear that the excess of nutrients provided in the diets would minimize the significance of differences that may have existed in net energy values of the hays.

The possibility of the differences in lactation and weight change responses being due to variations in the net energy values of the forages per unit of TDN showed a need for an evaluation of the productive value of the forages that would include maintenance, milk production, and changes in body weight. This

evaluation has been described by Knott *et al. (18)* and is determined by the following formula:

$$\% \text{ efficiency of utilization of available TDN} = \frac{\text{TDN requirements for maintenance and production}}{\text{TDN available (consumed TDN plus TDN equivalent of weight loss) or (consumed TDN} - \text{TDN equivalent to weight gain)}}$$

using 1 lb. of gain in body weight as equivalent to 3.53 lb. TDN and 1 lb. of loss in body weight as equivalent to 2.73 lb. TDN. The percentage efficiency of utilization of available TDN by roughage groups is shown in Table 4. After

TABLE 4

The percentage efficiency of utilization of available TDN, unadjusted and adjusted for TDN intake as per cent of requirements

Dietary groups	Unadjusted	Adjusted	Correlations
Alfalfa	113.1	113.6	−0.343
Sericea	113.0	108.7	−0.916
Service–urea	84.2	84.6	−0.902
L.S.D., P = 0.05	43.6	35.7	———

accounting for TDN gained or lost due to weight changes, the average utilization of available TDN by the alfalfa and the sericea groups was similar and averaged 29% greater than that of cows on the sericea-urea diet, but the differences were not statistically significant. Further study of the data revealed wide differences in percentage of efficiency of utilizing available TDN within feed groups, suggesting that some uncontrolled variable was affecting utilization of available TDN. Subsequently, other analyses were made in which the TDN intake during the experimental period, expressed as a percentage of requirements for maintenance and FCM produced during the standardization period, was used as a covariable with the per cent efficiency of utilization of the available TDN during the experimental period to determine whether or not the variations in efficiency of utilization of TDN were related to TDN requirements. The results of this statistical study showing the adjusted percentage of efficiency in utilizing available TDN are given in Table 4. Taking into account the level of TDN intake expressed as a percentage of the TDN requirements for maintenance and milk production significantly reduced the within-group variation in efficiency of TDN utilization, but had no appreciable effect on differences between dietary groups in efficiency of utilization of available TDN. The average efficiency of utilizing available TDN by the alfalfa-fed cows and that of cows fed sericea was 29.0 and 24.1% greater, respectively, than that of cows fed sericea-urea, but the differences were not significant statistically. The regression coefficients and the correlation coefficients between TDN intake as percentage of requirements and efficiency of utilizing available TDN associated with dietary groups differed significantly from the average coefficients for the experiment. The average correlations by dietary groups were: alfalfa, –0.343; sericea, –0.916; and sericea-urea, –0.902; whereas the average correlation for all diets

was –0.860, which is highly significant. These data show that overfeeding reduced the efficiency of utilization of available TDN and that efficiency was reduced significantly more by feeding excess TDN when sericea was the only roughage than when alfalfa was the only roughage fed. This reduction in efficiency of utilization of available TDN when the cows were overfed would be expected, since previous studies by Forbes *et al. (2)* have shown that rations have a higher net energy value for maintenance than for fattening.

Another statistical study of the efficiency of utilization of available TDN data was made by using the percentage of TDN supplied by hay as a covariable. These analyses showed that the within-treatment variation in percentage of efficiency in utilizing available TDN was not affected significantly by the variation in the percentage of TDN supplied in the form of hay. The correlation coefficients between percentage of TDN intake from hay and efficiency in utilizing available TDN by diets were: alfalfa, 0.493; sericea, –0.739; and sericea-urea, –0.892. The mean correlation for all diets was –0.324, which was nonsignificant, whereas that for the two sericea diets combined was –0.719, a significant correlation ($P \leq 0.05$). These correlation data indicate that increases in the quantity of sericea hay TDN fed affected adversely the efficiency of utilizing available TDN, whereas increases in the percentage of TDN supplied by alfalfa hay appeared to have no measurable effect on the efficiency of utilizing available TDN. Thus, it appears probable that sericea hay supplied some factor that depressed gross efficiency of milk production and, in its highest concentration, depressed efficiency of utilization of available TDN. It is known that sericea contains a higher percentage of tannin than alfalfa *(8)*; however, a study reported by Herman *et al. (12)* indicates that tannin does not affect milk yield. Also, a study by Hawkins *(8)* showed that the addition of gallotannin did not affect digestibility of dry matter or of protein. Hence, it appears improbable that tannin was the causative factor in the low productive value of sericea forage.

It is possible that the sericea forage was deficient in some nonenergy yielding nutrient, such as the unidentified lactation factor described by Huffman and Duncan *(14)* and Huffman *et al. (15)*, that was required to balance the ration. Forbes *et al. (3)* found that corn had a higher net energy value for steers when fed with alfalfa hay than when fed alone. The sericea had a higher productive energy value when the TDN supplied by the hay was less than 50% than when it exceeded 50% of the total. Since the highest percentages of TDN supplied by sericea hay and the overfeeding of TDN occurred with the same cows, the data do not lend themselves to an evaluation that would determine whether or not a deficiency of a nonenergy yielding nutrient existed. It is pointed out that the average apparent digestible protein content of sericea rations provided the minimum recommended allowance *(20)* of digestible protein and that the urea added increased the "digestible protein equivalent" of the SU diet to a level similar to that of the basal diet. Therefore, it would appear that digestible protein was not a limiting factor in the utilization of TDN or in milk yield of cows fed sericea forage. This concept is supported by the results of a later study in which additional digestible protein supplied by cottonseed

meal, as in the calf growth study *(10)*, did not sustain milk yield of cows receiving sericea.

The fact that the efficiency of utilizing available TDN by cows on the sericea diets was in excess of 100% of the expected efficiency *(18)* when the cows received the recommended allowance *(20)* or less than the recommended allowance of TDN shows that depression of lactation rather than depression of efficiency in utilizing available TDN was the primary inadequacy of these diets.

One cow fed sericea-urea developed a condition that was diagnosed as acetonemia. Two other cows on this diet developed enlarged briskets about 2 weeks after conclusion of the experiment. The latter cows had low blood serum levels of carotene and vitamin A. A priori relationships, however, have not been established.

SUMMARY

The results of this study show that the level of milk production of similar cows was less when sericea hay was the source of roughage than when good quality alfalfa hay was the roughage source even though the TDN intake on the two diets was similar. This difference in milk yield between the alfalfa and the sericea group was not overcome by supplying urea nitrogen to increase the "digestible protein equivalent" of the sericea ration to a quantity similar to that of the digestible protein content of the alfalfa hay (basal) ration.

Gross efficiency in milk production from TDN fed showed that cows receiving alfalfa utilized their TDN intake more efficiently for milk production than did cows fed sericea with or without urea as a supplement. A study of efficiency of utilizing available TDN that took into account maintenance, milk production, and gains or losses in body weight showed that sericea and alfalfa were similar in efficiency of utilization of available TDN. The average efficiency in utilizing available TDN was approximately 24% less by cows receiving sericea with urea than by cows receiving sericea without urea; however, this difference was not statistically significant.

Since efficiency of utilizing available TDN was similar for the alfalfa and the sericea groups, it is believed that the net energy of the two forages in proportion to their TDN also would be similar. Thus, it would appear that sericea contains a lactation depressant or that sericea is deficient in some nonenergy yielding nutrient required for high levels of milk production. Further studies appear to be warranted to determine whether the depressing effect of sericea on milk production can be counteracted by use of other feeds as a supplement.

It would appear that digestible protein was not a limiting factor in the quantity of milk produced. However, the possibility that something in sericea reduced the digestibility of the protein in the concentrate cannot be discounted. Supplementation of sericea with urea did not improve sericea as a roughage for dairy cows and there were some indications that it was deleterious.

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TECHNICAL NOTE

THE EFFECT OF ADMINISTERING 9-ALPHA FLUOROHYDROCORTISONE WITH SODIUM SOURCES TO RUMINANTS[1, 2]

In studies in Maryland *(6, 8)* it was observed that 9-alpha fluorohydrocortisone (9 – α F) is extremely effective for the treatment of bovine ketosis. No adverse results were observed in either normal or ketotic cows after its use except for a poor response in one case of ketosis, in which a massive dose of 300 mg. was administered. Later, however, in studies in the Los Angeles area, five deaths occurred in cows treated with 9 – α F, which were attributed to hypokaliemia *(8)*. Since these studies were in conjunction with studies on the use of various lactates, a check revealed that, in addition to 9 – α F, sodium lactate had been used in these cases as well as calcium lactate and sometimes calcium gluconate.

Studies on 9 – α F have shown a marked effect on sodium retention in the adrenalectomized dog *(4)* and rat *(2)*. A high degree of electrolyte activity, manifested by a decrease in the urinary excretion of sodium and a rise of potassium, was demonstrated with this substance in humans *(10)*. It was further demonstrated that this effect of 9 – α F was greater than that of cortisone *(3, 5, 9)*.

It appeared, therefore, that the adverse effect of 9 – α F noted in the Los Angeles studies may have been due to excessive sodium retention. The following is a brief report on administering 9 – α F with sodium and calcium sources to ruminants.

One Jersey cow and four goats were used in this study. The cow was used in two trials with a rest period of 3 weeks between trials. For 5 days prior to the beginning of Trial II (not noted in Table 1) this cow received 2 lb. of calcium lactate per os per day. As will be noted in Table 1, the animals received repeated injections of 9 – α F, alone and in combination with calcium lactate and/or sodium lactate per os and with calcium borogluconate intravenously.

The blood samples were drawn into tubes containing heparin. Sodium and postassium were determined with a flame photometer and calcium was determined by the Clark-Collip method (1).

The data in Table 1 show that the administration of 9 – α F with sodium salts had serious deleterious effects on all of the animals so treated. However, numerous injections without sodium salts were required before adverse clinical signs were produced and these signs disappeared rather quickly after cessation of the treatments. All four goats which received added sodium salts per os died within 2 days of the initial injection of 9 – α F. The cow treated similarly was prostrate repeatedly and was saved only by resorting to repeated intravenous injections of potassium chloride at short intervals. The signs and symptoms were the same as those observed earlier in the Los Angeles study, the cow exhibiting extreme flaccidity, rapid respiration, and rapid and sometimes irregular heart beats. The heart beat of the experimental cow following its first collapse was 80, strong and irregular. Within 15 hours it had increased to 110 and was quite weak though regular. Within 5 minutes after the intravenous injection of 10 g. of potassium chloride, the heart rate was 86, strong and regular. Several hours elapsed, however, before the cow regained sufficient muscle tone to lie in a natural position. Allowing a time lapse of 45 to 60 minutes between injections of potassium chloride usually resulted in a marked increase in heart rate.

The blood plasma potassium of the cow was depressed by 9 – α F injections alone, as was the blood and plasma potassium of the goat (Trial III) which received 9 – α F with calcium sources. A marked sodium retention was not apparent, however. In the other trials in which sources of sodium were administered, the injection of 9 – α F did result in both a marked depression of blood and plasma potassium and in apparent sodium retention as shown by the increase in plasma and/or blood sodium. Blood plasma calcium, where measured, was not affected by the various treatments.

It is obvious that sodium salts are contraindicated when 9 – α F is used for the treatment of bovine ketosis and that 9 – α F must be used with caution. However, since adverse effects have not been observed in either normal or ketotic cows on normal rations following the use of up to 100 mg. of 9 – α F, it may still have a real place in the treatment of bovine ketosis because of its great potency. It would be helpful to have more data on the effect of lower levels of sodium chloride intake.

In considering the use of 9 – α F for bovine ketosis, it is significant that a 100-mg. dose appears to be optimal for most cases and that, for the bovine, it is approximately 15 to 20 times as potent as hydrocortisone and 4 to 5 times as potent as prednisone *(6)*, both of the latter compounds being highly effective when administered in adequate amounts *(7, 8)*.

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TABLE 1 (Continued)

The effects on ruminants of intramuscular injections of 9-alpha fluorohydrocortisone with and without sodium salts per os

Trial	Treatment[a, b, c]	Treatment administered, blood (B) and plasma (P) substances in mg. % and clinical state of animals on selected days									
		Days									
		1	2	3	4	5	6	7	8	9	14
I Cow A	9 − α F	100 mg.	100 mg.	100 mg.	100 mg.			100 mg.	100 mg.	100 mg.	
	Blood analysis	K(P) 12 Na(P) 338			11 338 Normal	Normal	Normal	8 338 Normal	Resp. labored	9 358 Resp. labored	Normal
II	Ca L + Na L 9 − α F KCl intrav.	2 lb. 150 mg.	2 lb. 100 mg.	4 lb.	4 lb. 100 mg.			b	b	b	
	Blood analysis	Ca(P) 10 K(P) 13 Na(P) 325			10 7 350 Normal		10 4 563 In coma	10 6 338 In coma	Improved	Adm. diuretic Improved	10 15 363 Recovered
III Goat A	Ca L Ca G intrav. 9 − α F	125 g.	125 g. 100 mg.	125 g. 100 mg.	125 g.	125 g.	125 g. 9 g. 100 mg.	125 g. 9 g. 100 mg.			
	Blood analysis	Ca(P) 14 K(P) 17 K(B) 108 Na(P) 363 Na(B) 250			— 10 78 375 275		— 17 80 363 263		14 9 76 375 263		

TABLE 1 (Concluded)

The effects on ruminants of intramuscular injections of 9-alpha fluorohydrocortisone with and without sodium salts per os

Trial	Treatment[a, b, c]	Treatment administered, blood (B) and plasma (P) substances in mg. % and clinical state of animals on selected days									
		Days									
		1	2	3	4	5	6	7	8	9	14
IV Goat A	NaCl 9 − α F		80 g.	80 g. 25 mg.	80 g. 25 mg.						
	Blood analysis	K(P) 23 K(B) 96 Na(P) 363 Na(B) 263			7 66 400 313		Died on day 5				
V Goat B	Na L 9 − α F		150 g.	150 g. 25 mg.	150 g. 25 mg.						
	Blood analysis	K(P) 18 K(B) 74 Na(P) 363 Na(B) 263			6 52 325 313		Died on day 5				
VI 2 goats C and D	Na L 9 − α F	75 g.	150 g. 25 mg.	150 g. 25 mg.			Both goats died on day 4				

[a] Calcium lactate (Ca L) and sodium lactate (Na L) were 1:1 mixture.

[b] A total of 269 g. KCl were injected intravenously in 54 hours to cow A, second trial, in small aliquots at intervals of 15 to 30 minutes.

[c] Calcium borogluconate (Ca G) was a 25.7% solution of calcium gluconate.

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We hope, while you are here, that you will examine our facilities. Your headquarters will be the air-conditioned Student Union, and I hope that you will explore it, our modern library, and other buildings on the campus.

We are eager to make your visit to Oklahoma very pleasant and profitable. If there is anything we may do to assist you, please do not hesitate to let us know.

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Public Relations

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5 E. Long Street, Columbus 15 (1959)

P. R. ELLSWORTH, Milk Industry Foundation, 1145 Nineteenth Street, N. W., Washington, D. C. (1957)
J. HOFFMAN ERB, The Borden Company, 165 N. Washington Avenue, Columbus, O. (1958)
D. H. JACOBSEN, American Dairy Association, 20 N. Wacker Drive, Chicago (1957)
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D. M. SEATH, Univ. of Kentucky, Lexington

Student Affiliate

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FARNUM M. GRAY, Southern Dairies, Inc., 500 Dalton Avenue, Charlotte, N. C.

World Dairy Congress Federation

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Sub-Committee to Develop a Score Card for Nonfat Dry Milk

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R. J. REMALY, American Dry Milk Institute, Chicago, Ill.

F. E. Fenton, USDA, Washington, D.C.
B. M. Zakariasen, Land O' Lakes Creameries, Minneapolis, Minn.
N. P. Tarussuk, Univ. of California, Davis
W. K. Stone, Virginia Polytechnic Institute, Blacksburg
Ray Powers, Borden Co., New York, N.Y.

Score Card for Cottage Cheese

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S. L. Tuckey, Univ. of Illinois, Urbana
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L. H. Harmon, Michigan State Univ., East Lansing
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T. D. Harmon, Ohio State Univ., Columbus
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C. C. Olson, North Dakota State College, Fargo (1957)

Resolutions

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W. R. Hesseltine, Univ. of Connecticut, Storrs (1958)
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4-H Club

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Nevels Pearson, Michigan State Univ., East Lansing (1958)
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H. L. Mann, Pennsylvania State Univ., University Park (1959)

VOLUME XL January, 1957 NUMBER 1

JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

BOOK REVIEWS

1. Prentice-Hall Federal Tax Course, 1957. Prentice-Hall, Inc., Englewood Cliffs, N. J. Price — $17.50.

A 1518 page treatise dealing with answers to tax questions, tax savings, and new regulations under the new code, written in a clear and concise manner. Detailed examples are given to illustrate various tax problems. The book is excellently indexed for quick reference. The subject is treated from the viewpoint of the individual, partnerships, estates, trusts, corporations, foreign tax payers, social security taxes, gift taxes, and estate taxes. A book of this type is indispensable for the business as well as the professional man who has any appreciable involvement in tax problems.

P. H. Tracy

2. How to Win Success Before 40. W. G. DAMROTH. Prentice-Hall — publishers. Price — $4.95.

This book while slanted towards the young man in business could be of value to a person in scientific and professional fields as well. Twenty-one keys to success are covered in detail under three sections as follows:

Part I. How to improve your thinking pattern.

This section tells you how to choose a course in life and how to set up goals. It also explains how to develop self-confidence.

Part II. How to improve your working pattern.

This section covers such points as finding the kind of work you like, the need for a positive attitude, the need for continuing education, and how to take advantage of opportunities experienced.

Part III. How to improve your relations with other people.

This section has to do largely with how to sell yourself and your ideas to others.

Forty stories dealing with the actual experiences of young men and women who have achieved success in the last few years are given to prove the author's point that there is still ample opportunity for young people to arrive at the top today if they apply themselves properly. It is the purpose of the book to show how this can be done. It is stimulating reading.

P. H. Tracy

CHEESE

3. The influence of environment and processing of spoilage organisms in cottage cheese. L. G. HARMON and C. K. SMITH, Depts. of Dairy Microbiology and Public Health, Mich. Agr. Exp. Station, East Lansing. J. Milk and Food Technol., 19: 252. 1956.

Microbiological studies were performed on 17 vats of cotage cheese manufactured in 12 commercial plants. Samples of all raw materials and the cheese at various stages of manufacture were examined. Samples from 10 vats held at 50° F. exhibited a shelf life ranging from 5 to 10 d., while that of 7 vats ranged from 13 to 16 d.

The shelf life of samples at 42° F. averaged 51% longer than those samples held at 50° F. The pH of the cheese and temperature at which the product was washed and handled are important factors in keeping quality.

Many water samples showed high lipolytic and proteolytic microbial counts. No coliforms were reported in the water samples.

The presence of spoilage organisms in cheese samples indicates a need for proper pasteurization, equipment sanitation, precaution in handling raw materials, and lastly, the wash water should not be overlooked.

H. H. Weiser

4. Mold free cheese. Anon. Milk Prod. J., 47, 10: 83. 1956.

The antibiotic cure and preservation of cheese is accomplished by spraying or dipping the surface of freshly-made cheese with a weak antibiotic solution. A coat of paraffin then is applied to prevent evaporation of moisture. The protective antibiotic coat prevents mold growth and allows higher ripening temperatures. This process permits the ripening of cheese in 3 mo. instead of the usual 9-12 mo.

This process has been patented and is awaiting Food and Drug Administration approval.

J. J. Janzen

5. The use of the A-C test in Cottage Cheese manufacture. S. W. Reid. Milk Prod. J., **47**, 10: 26. 1956.

One of the most important steps in the manufacture of cottage cheese is determining the exact optimum time for cutting the curd. Techniques used for determining cutting time have been the "clean break" method, titration of whey against a known alkaline solution, and pH determinations of curd.

A new test, recently proposed by the Department of Dairy and Food Industry, University of Wisconsin, known as the A-C (Acid Coagulation Endpoint) test is described in detail.

J. J. Janzen

CONDENSED AND DRIED MILKS; BY-PRODUCTS

6. Acceptability of irradiated foods. V. E. McGary and M. E. Shipman, Med. Nutrition Lab., Fitzsimons Army Hospital, Denver. J. Am. Dietetic Assoc., **32**, 11:1059. 1956.

Thirty-six irradiation-sterilized foods, including powdered whole milk, were tested on nine or ten subjects. The test foods were incorporated in regular menus at different levels. Powdered whole milk was among those foods to which there were greatest flavor objections. It also acquired a yellow or tan cast on irradiation.

F. E. Rice

7. Importance of rapid cooling of nonfat dry milk. T. I. Hedrick and B. J. Ommodt, Agr. Mkt. Ser., USDA. Milk Prod. J., **47**, 10: 18. 1956.

Nonfat dry milk should be cooled immediately to 90 to 100° F. upon discharge from the drier to prevent lumpiness and caking. The cooling rate of nonfat dry milk in drums and closely stacked bags is very slow, especially during warm weather. In addition to lumpiness and caking, the development of off-color, stale and storage flavors are greatly accelerated.

Types of dry milk cooling systems that have been tried include atmospheric air, refrigerated air, refrigerated surfaces, and dry ice. Air at atmospheric temperature is the least effective during warm weather. Metal contact cooling is limited because during operation moisture collects on the cooled surface despite preventive efforts. Dry ice has been used with limited results, especially for high fat dry milk. The expense and inconvenience of handling prohibits its use for low price products such as nonfat dry milk. The most common system is the use of cooled air.

J. J. Janzen

8. New way to instantize milk solids. E. Carlson, R. A. Wier, and J. V. Ziemba. Food Eng., **28**, 10:62. 1956.

This report describes the Cherry-Burrell System of producing "instant" milk powder at Dry Milk Products Company, Eau Claire, Wisconsin. Starting with conventionally dried milk, the process involves agglomeration of the powder by dampening and clumping the particles, drying in hot air, shaker cooling, and finally sizing to uniform pellets ready for packaging. The different units of the equipment are described and processing steps are presented in detail. Capacity is 2000 lb. powder/hr.

T. J. Claydon

9. New twists in lactose uses. Food Eng. Staff. Food Eng., **28**, 10:56. 1956.

Lactose is being recognized by food manufacturers as an important aid in improving products and cutting ingredient costs. It possesses certain quality promoting properties combined with low sweetening power. The use of lactose is of value in controlling formulations, aiding processing techniques, simplifying product handling, improving products and producing new products. Lactose is commercially produced by crystallization from concentrated whey, under carefully controlled conditions.

T. J. Claydon

DAIRY BACTERIOLOGY

10. Vitamin assay. Vitamin B_6 in sterilized milk and other milk products. A. Z. Hodson, Research Lab., Pet Milk Co., Greenville, Ill. J. Agr. Food Chem., **4**: 876. 1956.

Two microbiological assay methods for vitamin B_6 have been slightly modified to conform to the newer knowledge of the vitamin and the metabolic requirements of the assay organisms. The modified methods give similar but not necessarily identical results when applied to fresh or pasteurized milk or nonfat dry milk solids, but different results when applied to stored evaporated milk. Experimental work suggests that pyridoxal of fresh milk is changed progressively to pyridoxamine and then to an unknown form of vitamin B_6 during processing and storage of evaporated milk. The unknown form of the vitamin is not equally active for the assay organisms.

S. Patton

11. Ruminant nutrition. Phytate phosphorous hydrolysis and availability to rumen microorganisms. A. Raun, E. Cheng, and W. Burroughs, Iowa State Coll., Ames. J. Agr. Food Chem., **4**: 869. 1956.

Calcium phytate is available to rumen microorganisms as inorganic phosphorus when tested by an artificial rumen technique involving cellulose digestion. Washed suspensions of rumen microorganisms were able to hydrolyze appreciable amounts of calcium phytate as measured by

the presence of inorganic phosphorus following incubation. The optimum pH for phytase activity was found to be approximately 5.5. Additional factors influencing phytase production or activity were concentration of microorganisms in the medium, time of incubation, and substrate concentration.

S. Patton

12. The characteristics of some rumen lactobacilli. R. G. JENSEN, K. L. SMITH, J. E. EDMONDSON and C. P. MERILAN. J. Bacteriol., 72: 253. 1956.

Of 168 isolates of lactobacilli from rumen fluid (2 from a sheep and the remainder from a cow on various occasions), 153 were identified. 12 were *Lactobacillus acidophilus*, 5 *L. brevis*, 30 *L. bruchneri*, 4 *L. casei*, 46 *L. casei* (nonlactose fermenting), 6 *L. fermenti* and 52 *L. plantarum*. All types except *L. casei* and *L. acidophilus* were found in rumen fluid dilutions of 10^{-6} or 10^{-7}.

F. E. Nelson

13. Characteristics of two new genera of anaerobic curved rods isolated from the rumen of cattle. M. P. BRYANT and N. SMALL. J. Bacteriol., 72: 22. 1956.

The genus *Succinivibrio* is suggested to include anaerobic, gram negative, non-sporeforming curved rods with monotrichous polar flagellation. These organisms ferment glucose with the production of a large amount of succinic acid and considerable acetic acid. The genus is placed in the tribe *Spirilleae* of the family *Pseudomonadacea*. *Succinivibrio dexterinosolvens* nov. sp. is the name proposed for the type species. This species is described in detail. Seven strains were studied, all varying to some small degree from the type culture. These organisms are found in greater numbers in the rumen of grain-fed cows than in the rumen of cows fed predominantly on roughage. These organisms produce no appreciable amounts of methane, hydrogen, butyric acid, propionic acid or volatile alcohol.

The generic name *Lachnospira* is proposed for five strains of anaerobic, gram positive nonsporeforming, monotrichous, curved rods. The genus is tentatively placed in the tribe *Spirilleae* because of morphology, but the gram reaction, which is relatively weak, does raise a question about placement. *Lachnospira multiparus* nov. sp. is proposed as the name for the type species. Glucose is fermented to give H_2, CO_2, ethanol and acetic, formic and lactic acids. No methane, or butyric, propionic or succinic acids were produced. These organisms frequently are present in considerable numbers in rumen contents, but they never represent a major fraction of the strains isolated from a given sample of rumen fluid. Presumptive identification may be made on the basis of the characteristic filamentous colony.

F. E. Nelson

14. The anaerobic monotrichous butyric-acid producing curved rod-shaped bacteria of the rumen. M. P. BRYANT and N. SMALL. J. Bacteriol., 72: 16. 1956.

A new genus *Butyrivibrio* is suggested for a group of rumen bacteria that are monotrichous, nonsporeforming, gram negative curved rods that ferment glucose with the production of large amounts of butyric acid. The genus was placed in the tribe *Spirilleae* of the family *Pseudomonadaceae*. Among the 48 cultures examined, there was considerable variation in morphological and physiological characteristics. The type species, *Butyrivibrio fibrisolvens* nov. sp., was described upon the basis of the characteristics of one of the most representative strains. This species produced CO_2, H_2, and butyric, formic and lactic acids from glucose. Many other carbohydrates also are fermented. Other members of the genus not placed in the type species differ mainly in not fermenting some of the carbohydrates. One of the most different strains did not produce H_2 and produced large amounts of formic acid.

These organisms appear to be among the most numerous types in the rumens of cattle and sheep fed various rations. They digest many of the major constituents of ruminant rations, such as xylan, celluiose, starch and protein.

F. E. Nelson

15. Proteolysis by Lactobacillus casei. I. Proteinase activity. E. BRANDSAETER and F. E. NELSON. J. Bacteriol., 72: 68. 1956.

Maximum proteolytic activity of the cell-free extract was at pH 5.5 to 6.0, with more than half of the activity remaining at pH 5.0. Addition of $CaCO_3$ to a milk culture caused a marked increase in proteolysis, probably because the reaction was maintained above pH 5.0. Maximum casein degradation by the cell-free extract in a 30 hour period was obtained at 50° C. and pH 6.0. The enzyme activity showed maximum heat stability at pH 5.0 to 5.5. The characteristics of the enzyme indicate that *L. casei* may be an important factor in the protein degradation in Cheddar cheese.

F. E. Nelson

16. Proteolysis by Lactobacillus casei. II. Peptidase activity. E. BRANDSAETER and F. E. NELSON. J. Bacteriol., 72:73. 1956.

The cell-free extract of this organism showed activity against a number of di- and tri-peptides. Using a reaction time of 1 hr. at pH 7.0, maximum activity on most peptides was obtained at 45° C. Cobalt at 0.0004 M concentration increased peptide hydrolysis at pH 5.5 considerably in many cases and also increased hydrolysis of glycylglycine at pH 7.7. Activation by Zn^{++} was greatest at pH 7.5-8.5. An equimolar concentration of Zn^{++} prevented peptidase activation by Co^{++} at pH 5.5. The peptidase activities at pH 5.7 in the presence of

Co^{++} were less stable toward heat than were the peptidase activities at pH 7.0 to pH 8.0 in the absence of Co^{++}.

F. E. Nelson

17. The utilization of dihydrothymine by Streptococcus faecalis in the absence of folic acid and thymine. J. F. Walper and R. M. Fink. J. Bacteriol., 72:105. 1956.

Dihydrothymine supported growth of *S. faecalis* in a medium deficient in both folic acid and thymine. The activity was much less than that of thymine or folic acid. The action of dihydrothymine apparently was not due to the presence of thymine.

F. E. Nelson

18. The acetate requirement of Streptococcus faecalis. G. D. Shockman. J. Bacteriol., 72:101. 1956.

Under conditions of good aerobiosis, adequately depleted inoculum and omission of reducing agents in the culture medium, an absolute requirement for acetate exists. Under anaerobic conditions, no such requirement exists. Lipoic acid plus thiamine may substitute for acetate under aerobic conditions. Cells grown anaerobically or aerobically in the presence of acetate contain almost no lipoic acid. Cells grown aerobically on lipoic acid contain significant quantities of lipoic acid.

F. E. Nelson

19. The effect of carbon dioxide on polysaccharide production by Streptococcus bovis. J. A. Dain, A. L. Neal and H. W. Seeley. J. Bacteriol., 72:209. 1956.

The majority of strains tested required CO_2 for slime formation on sucrose-gelatin agar. Slime production in presence of CO_2, inability to ferment mannitol and greening on horse-blood agar were correlated in most instances. The CO_2 requirement could be replaced by including 0.1% Tween 80 in the plates and incubating anaerobically. The CO_2-dependent slime from one strain was identified as a glucose-containing polysaccharide.

F. E. Nelson

20. The role of succinate as a precursor of propionate in the propionic acid fermentation. H. G. Wood, R. Stjernholm and F. W. Leaver. J. Bacteriol., 72: 142. 1956.

Washed cells of *Propionibacterium arabinosum* and C^{14}-labelled carbon compounds were used. Propionate is not an inert end product of the propionic acid fermentation. Succinate was converted to propionate and acetate, but there was no evidence of a central direct cleavage of the succinate to two acetate molecules. The isotope carrier technique indicated propionate was converted to succinate very slowly, but among intracellular acids propionate is converted to succinate very rapidly. More than one pool of propionate and succinate exists in the cell and the conclusion is reached that succinate and propionate probably are formed by more than one pathway.

F. E. Nelson

21. Sporulation requirements of Bacillus coagulans var. thermo-acidurans in complex media. M. Amaha, Z. J. Ordal and A. Touba. J. Bacteriol., 72: 34. 1956.

Addition of $NiSO_4$, $MnSO_4$ or $CoSO_4$ at 1 ppm. to a peptone-containing agar medium markedly stimulated growth and sporulation. The effect was particularly pronounced at 55° C. incubation. Addition of malic or α-ketoglutaric acid to broth shake cultures containing manganese stimulated sporulation by one difficult strain. The role of manganese did not seem specifically related to stimulation of proteinase production by this element.

F. E. Nelson

22. Stimulation of growth initiation by heat degradation products of glucose. H. H. Ramsey and C. E. Lankford. J. Bacteriol., 72:511. 1956.

Lactobacillus fermenti was used as test organism. A solution of 20% glucose plus 2% phosphate (pH 6.4) and autoclaved at 121° C. for 15 min. gave more rapid initiation of growth when added to a complex basal medium than did glucose autoclaved in the medium, autoclaved alone or sterilized by filtration. The effect was best with autoclaving at pH 6.4, being much less above pH 7.0 and decreasing more gradually at acid pH values. Although autoclaving of glucose plus phosphate for 5 min. at 115° C. gave some effect over separately autoclaved glucose, 15 or 60 min. at 121° C. gave larger and more numerous colonies. The initial stationary phase was inversely related to the glucose concentration supplied from the glucose-phosphate mixture. The effective agent apparently is neither an aldehyde or ketone nor a phosphorylated derivative of glucose. Partial purification has been achieved by column chromatography. A number of other gram positive bacteria also initiated growth more promptly in the presence of autoclaved glucose-phosphate mixture, while a number of gram negative organisms tested were not affected.

F. E. Nelson

23. Discovery of of a new acetate-replacing factor. H. R. Sheggs, L. D. Wright, E. L. Cresson, G. D. E. MacRae, C. H. Hoffman, D. E. Wolf, and K. Folkers. J. Bacteriol., 72:519. 1956.

Lactobacillus acidophilus strain 4963 was used as test organism, but *L. bifidus*, *L. bulgaricus* and *Thermobacterium acidophilus* also responded. A "vitamin B_{12}" concentrate subjected to a 200-plate countercurrent distribution between ether and water yielded both a lipoic acid fraction and the new acetate-replacing factor. Commercial whey, fish solubles, distillers' sol-

ubles and certain yeast extracts are relatively rich sources of the factor. An assay procedure and a procedure by which useful concentrates of the factor may be obtained are outlined.

F. E. Nelson

24. Some characteristics of a growth stimulant in corn steep liquor for Lactobacillus casei. A. H. HEIMBUCH, L. W. AURAND and M. L. SPECK. J. Bacteriol., 72:543. 1956.

Chromatographic studies, absorption spectrum in the ultraviolet region and chemical stability characteristics indicate the factor is a nucleoside, probably of pyrimidine. The factor is different from others described in the literature for this organism.

F. E. Nelson

25. Culture and physiology of a starch-digesting bacterium (Bacteroides amylophilus n. sp.) from the bovine rumen. L. J. HAMLIN and R. E. HUNGATE. J. Bacteriol., 72:548. 1956.

This organism is gram negative and nonmotile, in addition to digesting starch. Pleomorphism occurred to varying degrees with the different strains and large intracellular granules could be detected in large cells. Of a considerable number of hexoses and disaccharides tested, only starch and maltose were fermented. Acetic, formic and succinic acids are produced. Traces of ethanol and lactic acid may be formed. These organisms were not found at all in three animals examined and were not always found in the rumens of the three animals from which isolations were made. Occasionally they are present in relatively large numbers. Methods of study are well presented.

F. E. Nelson

DAIRY CHEMISTRY

26. The copper promoted decomposition of vitamin B_{12} in solutions of ascorbic acid. A. J. ROSENBERG, Merck and Co., Inc., Rahway, N. J. J. Biol. Chem., **219**, 2: 951. 1956.

Vitamin B_{12} is decomposed in solutions of ascorbic acid. The decomposition is strongly promoted by cupric ions which are inactivated in the process. Dehydroascorbic acid also promotes the decomposition of B_{12} in a similar manner to ascorbic acid. A study of the kineties of the reaction at 76.6° C. was made and a cobalamin-like product was recovered from the reaction.

B. L. Larson

27. A pressor material produced by the action of pepsin on casein. O. H. MCGLORY, N. S. OLSEN, and L. FIELD, Vanderbilt Univ., Nashville, Tenn. J. Biol. Chem., **219**, 1:299. 1956.

The formation of a pressor material from the action of pepsin on casein was confirmed. This material was isolated by a method suitable for large scale work and was purified to free it from substances having a depressor action. The material is called pepsitensin and is a polypeptide or mixture of polypeptides produced by true enzymatic action rather than autolytic.

B. L. Larson

28. Lactose metabolism. IV. The function of uridine triphosphate in the interconversion of glactose-1-phosphate and glucose-1-phosphate. R. G. HANSEN and R. A. FREEDLAND, Univ. of Illinois, Urbana. J. Biol. Chem., **216**, 1: 303. 1956.

Crude extracts from *L. bulgaricus* and *S. fragilis* interconvert galactose-1-phosphate and glucose-1-phosphate when catalytic amounts of either uridine diphosphate hexose or uridine triphosphate are present. Fractionated extracts respond only to uridine diphosphate hexose. Uridine diphosphate hexose was formed from either galactose-1-phosphate or glucose-1-phosphate, and uridine triphosphate from enzymes present in the *S. fragilis* extracts and in the unfractionated *L. bulgaricus* extracts.

B. L. Larson

29. Lactose metabolism. V. The uridine nucelotides in galactose toxicity. R. G. HANSEN and R. A. FREEDLAND, Univ. of Ill., Urbana. J. Biol. Chem., **219**, 1: 391. 1956.

By means of a formic acid-Dowex-formate column, uridine nucleotides were isolated from normal chicks and chicks fed galactose. Chicks fed galactose contained more than twice as much uridine diphosphate hexoses (UDPHexose) in their livers as normal chicks. In normal tissues glucose is the most abundant hexose found in UDPHexose although galactose is also present. In chicks with galactose toxicity, the concentration of UDPHexose is increased and in some cases galactose may be practically the only hexose in the UDPHexose fraction.

B. L. Larson

DAIRY ENGINEERING

30. New milk separation system ups quality, cuts cost. Anon. Milk Prod. J., 47, 10: 28. 1956.

A new "liquid seal" type separator has resulted in the production of better butter and cream from sweet milk as well as important cost-cutting advantages.

The liquid seal is simple in principle. The milk filled separator bowl rotates around a stationary "centripetal pump" (often referred to as a collector) which is built into the top of the machine. The pump disc periphery is submerged in the milk, creating the "liquid seal." The sealing action of this method is absolutely airtight and sanitary.

The liquid seal also provides pressure. The rotating milk or cream, in seeking exit, flows through the disc channels of the stationary centripetal pump (this is the reverse principle of a centrifugal pump) and the rotating energy is converted into pressure. The product is then

discharged foamlessly at practically any required pressure, thus eliminating the need for accessory pumps.

This separator can be used both for hot or cold separation of milk and whey.

J. J. Janzen

DAIRY PLANT MANAGEMENT AND ECONOMICS

31. Plastic body route trucks. Anon. Food Eng., 28, 10: 88. 1956.

New plastic body route trucks operated by Carnation Company possess several unusual features. They are both integrally insulated and refrigerated. Weight is 500 lb. less than standard metal bodies. They are easier to keep cool and are simpler to clean and sanitize. Although present initial cost is 6-10% higher than metal bodies this is more than offset by savings in operational costs, maintenance, and repairs.

T. J. Clayton

GENETICS AND BREEDING

32. Underdeveloped mandible in a herd of dairy Shorthorn cattle. H. T. Grant, Univ. of Nottingham, Sutton, Borrington, Loughborough, England. J. of Heredity, **47**, 4: 165. 1956.

The condition of a shortened mandible (2.5 cm. in one case) with molars impacted in both jaws was found in 15 cases ((11 males and 4 females) carried full term. Of 4 other defective calves born during this period, 1 was blind in both eyes, 1 had short forelegs, and two were not described. Six affected calves were first gestations and one or more represented gestations No. 2, 3, 4, 5 and 8. The defective calves were born over the period 1941-53 out of a total of at least 1202 calvings by 48 bulls. The 15 defective calves came from 12 different cows, only one of which produced more than one (4) defective calf. The parents of all 15 calves and of many others, traced one or more times to a bull that was twice a first prize winner at the Royal Show. The author suggested that a recessive pleiotropic jaw shortening gene could cause this and other similar defects variously called agnathia, brachygnathia, micrognathia, bird face, parrot mouth, parrot beak, overshot jaw, undershot jaw, fish face, fish jaw and pig jaw.

L. O. Gilmore

NUTRITIVE VALUE OF DAIRY PRODUCTS

33. Colic in infants. A nine-author panel discussion, led by J. Glaser, Univ. of Rochester School of Med. Pediatrics, **18**, 5:828. 1956.

The problems of defining "colic" and determining its causes are reviewed. Sensitivity to foods is not always the cause. One author (L. Breslow) studied 90 infants who had "the usual manifestations of colic," and listed 11% as having an intolerance for butter fat; 10% were classed as cases of "cow's milk allergy" (probably referring to protein). B. Ratner reported that he is "not entirely in accord with the use of milk substitutes unless the infant is sensitive to the casein fraction." Heat denaturing of milk and other foods reduces the incidence of colic in infants, when such condition has a basic allergic cause. There was general agreement among the authors that an allergic etiology accounts for only a small fraction of the colic seen in infancy.

F. E. Rice

34. Effect of added lysine on growth of rats fed a cereal and milk diet. H. P. Sarett, Mead Johnson Research Lab., Evansville, Indiana. J. Nutrition, **60**, 1:129. 1956.

A mixed cereal, Pablum, was used containing wheat, oats, and corn, with other added nonprotein constituents. The basal rat diet consisted of 70% Pablum mixed with 30% powdered whole milk, which was calculated to furnish 60% of the total protein as cereal and 40% as milk. Lysine content of this diet by calculation is 1.08%. On this basal diet the rats grew as well as they do on "stock diets." The addition of 0.14% or 0.28% of L-lysine did not significantly increase the gain in weight of the rats. The authors postulate that in infant nutrition the commonly fed mixture consisting of 61% cereal and 39% powdered milk, the equivalent of one part cereal to 5 parts of fluid milk, which contains about 1.2% lysine is satisfactory with respect to lysine content, and that there is no need to supplement such a diet with added lysine.

F. E. Rice

35. Weight gains in the first four weeks of infancy: A comparison of three diets. R. T. Gross, and L. E. Moses, Stanford School of Med. Pediatrics, **18**, 3:362. 1956.

The weight gains of 407 normal full term infants during the first 4 weeks of life are reported. Infants were fed as follows: 65 breast fed; 180 on an evaporated milk formula with added carbohydrate; 162 on a commercial infant food prepared to simulate average human milk in composition. Weight gain alone was taken as a criterion. Analyses of the data showed that there were no significant differences in weight gain between the three groups. The authors caution against too sweeping conclusion being drawn from this study since they made no observations or comparisons as to frequency of constipation or "colic," possible superiority in the breast fed group in part due to the ability and willingness of the mothers to feed at breast for four weeks, etc.

F. E. Rice

36. Studies of tocopherol deficiency in infants and children. H. M. Nitowsky *et al.*, Sinai Hospital, Baltimore. Am. J. Diseases of Children, **92**, 2:164. 1956.

Plasma tocopherol levels in postpartum mothers were 1.07 to 1.56 mg. % and in newborn infants 0.22 to 0.26 mg. %. Normal adults average 0.75 mg. %. All observations pointed to the conclusion that the placents is relatively impermeable to tocopherol and that there is difficulty in attempting to raise fetal vitamin E levels by administration of tocopherol to the mother. At average age of 46 days, after feeding cow's milk mixtures, mean plasma tocopherol levels rose to 0.33 mg. %, and after breast feeding, to 0.72 mg. %. Oral supplementation with small doses of tocopherol esters caused prompt rises in plasma tocopherol in the infants.

F. E. Rice

37. Milk powder for school children. J. Am. M. A. Foreign Letters. **168**, 8:830, 1956.

Observations were made on 337 scholo boys, some of whom were given non-fat dry milk obtained from United Nations Fund. One oz. of the powder per day in addition to the regular diet caused the group so fed to increase 0.4 in. in height and 0.63 lbs. weight over the non-milk group in one year.

F. E. Rice

38. Gastrointestinal allergy to cow's milk in the neonatal period. R. B. TUDOR, Bismarck, N. D., Hospitals. Lancet, **76**, 9:245, 1956.

Seven cases believed to be gastrointestinal allergy are reported. Six cases occurred on evaporated milk, and one on dry milk formulas. Symptoms were colic, vomiting, diarrhea, nasal stuffiness, cough, and/or wheezing. Five cases improved when placed on Mull-Soy, one on goat's milk, one on a meat base formula. The author reports that one baby gave a positive skin test to cow's milk. It is not stated if the diagnoses were checked by skin tests in the other cases.

F. E. Rice

39. Bottle-feeding supplement. E. WALCH, Heidelberg, Germany, Modern Med., **24**, 20: 256. 1956.

The addition of beta-methyl-N-acetyl-D-glucosaminide to a cow's milk formula fed to newborn babies results in a predominant *Lactobacillus bifidus* intestinal flora as in babies on human milk. And the stools resemble those of breast fed babies in consistency and color, and in containing almost exclusively gram-positive flora.

F. E. Rice

DIFCO

Bacto-Dehydrated Media
for Microbiological Assay of

VITAMINS and AMINO ACIDS

These media contain all the necessary nutriments for the growth of specified test organisms for the microbiological assay of vitamins and amino acids except for the component under assay. These basal media require only the addition of specified increasing amounts of the vitamin or amino acid being assayed to obtain a growth response which may be measured by acidimetric or turbidimetric methods for the construction of standard curves. The vitamin or amino acid content of the material under assay is determined by adding appropriate concentrations of the test substance to the basal medium and comparing the growth response obtained with that of the standard.

BACTO-RIBOFLAVIN ASSAY MEDIUM
BACTO-NIACIN ASSAY MEDIUM
BACTO-THIAMIN ASSAY MEDIUM
BACTO-PANTOTHENATE ASSAY MEDIUM
BACTO-B_{12} ASSAY MEDIUM USP
BACTO-CS VITAMIN B_{12} AGAR
BACTO-FOLIC ACID ASSAY MEDIUM
BACTO-PYRIDOXINE ASSAY MEDIUM
BACTO-BIOTIN ASSAY MEDIUM
BACTO-CHLORINE ASSAY MEDIUM
BACTO-CF ASSAY MEDIUM
BACTO-TRYPTOPHANE ASSAY MEDIUM
BACTO-LEUCINE ASSAY MEDIUM
BACTO-METHIONINE ASSAY MEDIUM
BACTO-LYSINE ASSAY MEDIUM
BACTO-ISOLEUCINE ASSAY MEDIUM
BACTO-ARGININE ASSAY MEDIUM
BACTO-TYROSINE ASSAY MEDIUM
BACTO-CYSTINE ASSAY MEDIUM

The method employed in carrying stock cultures of the test organisms and preparing the inoculum for microbiological assay is important. The following media have been developed especially for carrying stock cultures and for preparation of the inoculum:

BACTO-MICRO ASSAY CULTURE AGAR
BACTO-MICRO INOCULUM BROTH
BACTO-B_{12} CULTURE AGAR USP
BACTO-B_{12} INOCULUM BROTH USP
BACTO-NEUROSPORA CULTURE AGAR

BACTO-VITAMIN FREE CASAMINO ACIDS, dehydrated, is an acid hydrolysate of vitamin free casein prepared especially for laboratories investigating microbiological assay of vitamins.

Descriptive literature available upon request

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